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SOME PHYSIOLOGICAL STUDIES OF GLOEOSPORIUM PERENNANS AND NEOFABRAEA MALICORTICIS[1]

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INTRODUCTION

In certain sections of the Pacific Northwest the control of perennial canker and northwestern anthracnose has become a problem of great economic importance to the apple industry. Perennial canker has been attributed to *Gloeosporium perennans* Zeller and Childs (*13*),[3][4] and northwestern anthracnose is caused by *Neofabraea malicorticis* (Cordley) Jackson. Both organisms are associated with cankers on the limbs and trunk of the apple tree, and both produce rots on the fruits.

Northwestern anthracnose is known to have been of economic importance since 1891. The causal organism was described in 1900 by Cordley (*6*) and also by Peck (*12, p. 21*), Cordley giving it the name of *Gloeosporium malicorticis*. In 1913 Jackson (*10*) described the perfect stage of the fungus, changing the name to *Neofabraea malicorticis*.

In 1925 Zeller and Childs (*13*) reported the occurrence of perennial canker and described as the causal organism *Gloeosporium perennans*. Later in the same year Fisher (*7*) reported evidence that this disease had been in the Northwest for at least 11 years.

There is great similarity in the cankers and also in the rots produced by the two organisms, and it is generally agreed that the two diseases must have existed side by side for a number of years without any suspicion that two organisms were concerned. It was when the measures recommended for the control of anthracnose were found to be ineffective under Hood River conditions that Zeller and Childs (*13*) discovered the new species in the cankers. The chief distinctions they made between the two organisms at that time were that the perennial-canker fungus is a wound parasite but perennial in its nature when once established, whereas the anthracnose fungus is able to infect the uninjured bark but does not spread beyond the limits of the first year's growth; that the anthracnose organism has more active diastatic power than the perennial-canker organism and has curved or hooked conidia in contrast to the generally ellipsoid spores

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[2] The writer is grateful to J. S. Cooley, of the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, and S. M. Zeller, of the Oregon Agricultural Experiment Station, for furnishing cultures of the fungi studied; and to Charles Brooks, of the Division of Horticultural Crops and Diseases, for helpful advice and suggestions.

[3] Reference is made by number (italic) to Literature Cited, p. 76.

[4] Since this paper was prepared, Nellie A. Brown, of the Division of Horticultural Crops and Diseases, has reported (*2a*) that *Gloeosporium perennans* is frequently absent in otherwise typical cankers, that other fungi as well as bacteria are frequently found, and that under proper conditions typical cankers can be produced by artificial inoculation with these organisms as readily as with *G. perennans*.

of *Gloeosporium perennans*. More recently, however, Childs (*3*) has reported that the perennial-canker organism is not truly perennial, and Fisher and Reeves (*8*) have found evidence that it can produce cankers without the aid of previous injuries to the bark, thus reducing the number of well-established distinctions between the two organisms. But in spite of the great similarity of the two diseases, there seems to be no doubt that a new canker organism has appeared in the Northwest, and its control has become one of the outstanding problems in plant pathology.

The present work was undertaken with the hope that further information regarding the physiological responses of the two organisms might throw light on their relationship and also be of value in developing methods of control. Following out this idea, studies have been made of the effect of temperature, acidity, and nutrient salts upon the growth of the organisms.

EXPERIMENTAL PROCEDURE AND RESULTS

CULTURE MEDIA

The two canker organisms from the Northwest were grown on variously modified culture media. The canker organisms comprised authentic cultures of *Neofabraea malicorticis* and *Gloeosporium perennans* received from J. S. Cooley, at Hood River, Oreg., and three strains of each organism obtained from S. M. Zeller, of Corvallis, Oreg. The same methods were used in all the tests. Ten cubic centimeters of the medium were poured into a sterile Petri dish and allowed to solidify. Inoculations were then made in the center of the plate with either spores or mycelial tufts of the organism, and records of growth were taken at regular intervals.

It was found that all of the organisms could be satisfactorily grown on Dox's synthetic agar, so this was selected as the standard medium from which various modifications were made. The following is the standard formula for Dox's agar:

Distilled water	cubic centimeters	1,000
Magnesium sulphate	grams	0.5
Dipotassium phosphate	do	1
Potassium chloride	do	.5
Ferrous sulphate	do	.01
Sodium nitrate	do	2
Agar	do	15

The agar was prepared in three lots: (1) Dox's agar with 0.5 per cent dextrose; (2) as in 1, but lacking potassium chloride; (3) as in 1, but lacking sodium nitrate. When any one of the constituents of Dox's agar is omitted, it may be difficult to obtain a clear solution. To obviate this difficulty monobasic potassium phosphate (KH_2PO_4) was substituted for the dibasic potassium phosphate (K_2HPO_4). In other experiments potash was completely eliminated from the nutrients by substituting monobasic ammonium phosphate for the monobasic potassium phosphate besides omitting the potassium chloride.

The cultures were held at temperatures of 0°, 2.5°, 5°, 10°, 15°, and 20° C. in the refrigerator boxes described by Brooks and Cooley (*2*).

When the formula for Dox's agar contained either monobasic or dibasic potassium phosphate, the growth of the organisms was not inhibited by the absence of either potassium chloride or sodium nitrate

from the nutrients added to the agar. When monobasic ammonium phosphate was employed, both *Neofabraea malicorticis* and *Gloeosporium perennans* were moderately inhibited in their growth at 15° C. by the absence of sodium nitrate or potassium chloride from the nutrients, whereas at 5° and 10° only the lack of potassium chloride had any effect. (Fig. 1.) The rate of inhibition, however, was about as pronounced for one organism as for the other. The slight differences between the two are hardly significant, especially since there was always some variation in the rate of growth of the two organisms.

HYDROGEN-ION CONCENTRATION

A study was made of the responses of the anthracnose and perennial-canker organisms to different hydrogen-ion concentrations. Apple agar was prepared from ripe Delicious apples and the desired pH values were obtained by the addition of N/1 HCl or N/1 NaOH. A set of buffer solutions as designed by Clark and Lubs (*5*) were prepared for pH values ranging from 1.2 to 11.8. These were made

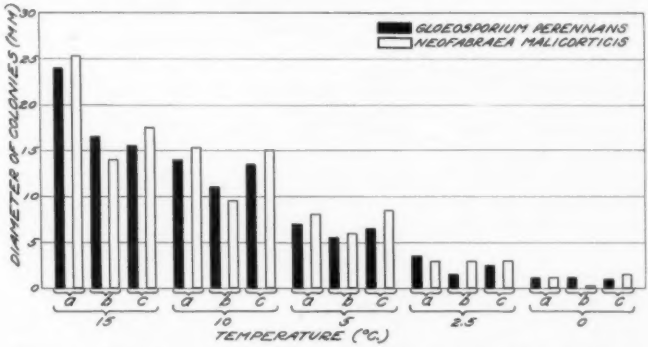

FIGURE 1.—Growth of *Gloeosporium perennans* and *Neofabraea malicorticis* at various temperatures on several modifications of Dox's agar in 20 days: *a*, Dox's agar plus 0.5 per cent dextrose; *b*, Dox's agar plus 0.5 per cent dextrose less KCl; *c*, Dox's agar plus 0.5 per cent dextrose less $NaNO_3$

up in strict accordance with the directions of Clark (*4*) both as to purity of chemicals and details of preparation. The buffers were standardized by means of Bunker's modification of the Hildebrandt bubbling hydrogen electrode, a calomel half cell, and a Leeds & Northrup type K potentiometer. A Bailey electrode was employed for determinations in some instances. The accuracy of the apparatus was determined by making readings on N/10 hydrochloric acid and M/20 potassium acid phthalate solutions.

Indicator solutions were prepared as directed by Clark (*4*), and by means of these and the buffers already mentioned a set of color standards was obtained covering a range of pH 1.2 to 10. For values higher than pH 10, two dyes, Tropaolin O and Alizarin G, were obtained from the Bureau of Chemistry and Soils. These dyes have been recommended by McCrumb and Kenney (*11*) for pH values 10 to 13. Although it was hoped to obtain pH values up to 12 or more, it was not deemed advisable to employ any higher than 11.8 because of the similarity of the color of the agar to that of the two dyes. The

pH values were determined colorimetrically by the use of the above-mentioned color standards for comparison.

Very acid or very alkaline agar will not solidify readily, and because of this fact the media having values under pH 3.8 or over 8.2 were sterilized first and the acid or alkali was added later by means of sterile pipettes. Those in the intervening range were combined with the approximate amount of acid or alkali and then sterilized in the autoclave with 10 pounds pressure for 10 minutes. The pH value was determined while the medium was still liquid. The agar was next poured into sterile Petri plates and when cool was inoculated in the center with spores. The plates were held for three weeks at 10° C., and the diameters of the colonies were measured at regular intervals. The results appear in Table 1 and in Figures 2 and 3.

TABLE 1.—*Growth of Neofabraea malicorticis and Gloeosporium perennans on apple agar of different initial pH values after three weeks at 10° C.*

Organism	Diameter of colonies (in millimeters) at pH of—																		
	2.0	2.2	2.4	2.6	2.9	3.0	3.6	3.8	4.0	4.1	4.8	9.3	9.5	9.7	9.9	10.0	11.2	11.3	11.8
Neofabraea malicorticis	0	3.3	4.5	8.0	14.5	24.5	22.0	28.5	28.5	29.0	29.0	28.5	30.1	30.0	30.1	29.6	30.8	29.3	24.6
Gloeosporium perennans	0	1.0	8.2	10.2	14.5	22.2	25.0	27.7	26.5	24.0	33.5	32.0	30.1	31.0	27.1	27.3	26.5	25.0	10.0

In the first of these experiments it appeared that the limiting pH value in the acid range lay between 2.0 and 3.6 for both organisms. Later it was found that the growth rate increased as the pH values were increased by 0.2 pH from 2.2 up to pH 3.0. From pH 3.0 up to pH 11.3 growth was normal. No growth was obtained at pH 2.0. At the alkaline end definite retardation was evinced at pH 11.8. This pH value appeared to exert a greater retarding effect on *Gloeosporium perennans* than on *Neofabraea malicorticis*, but the difference may be entirely experimental, because germination studies indicate that both fungi respond similarly in alkaline media. For instance, in some preliminary tests germination for both organisms was considerably reduced at pH 11.5 and there was no germination at pH 12.

Thus far the physiological responses of these two fungi (*Neofabraea malicorticis* and *Gloeosporium perennans*) have been shown to be very similar. Varying the nutrients in regard to potassium chloride or sodium nitrate did not affect the growth of one organism more than that of the other. The effects of different pH values on the rate of growth were similar for both organisms.

TANNIC ACID MEDIA

For further cultural studies of the two organisms a medium containing tannic acid was employed. Bavendamm (*1*) recommends a solid medium containing 0.5 per cent tannic acid for the purpose of detecting oxidases in wood-rotting fungi. Inasmuch as the advance of the colony is preceded by a dark ring, due to the oxidasic action, it was hoped that different rates of growth might be easily detected on this medium.

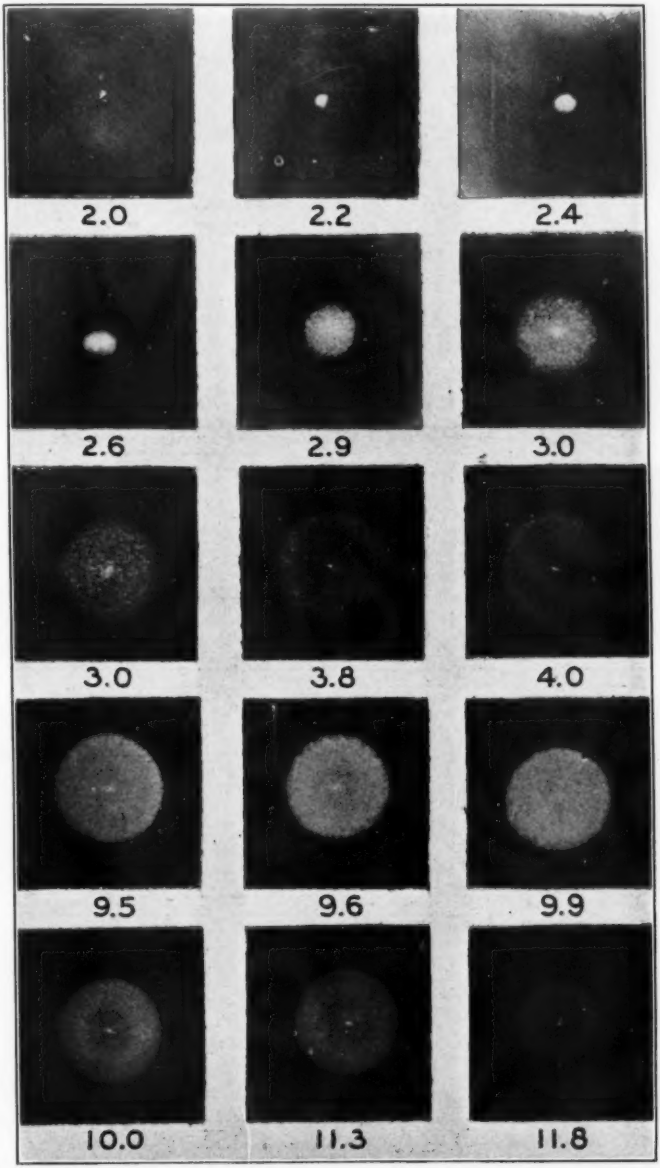

FIGURE 2.—Growth of *Neofabraea malicorticis* in 3 weeks at 10° C. on apple agar of 15 initial pH values from 2.0 to 11.8

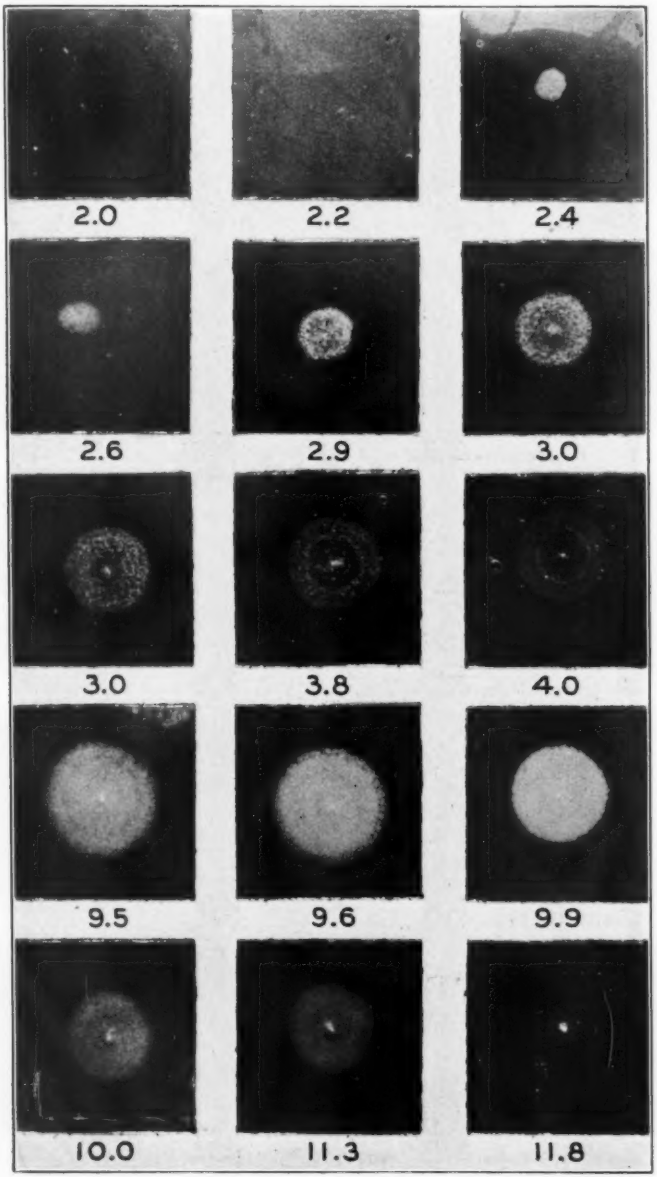

FIGURE 3.—Growth of *Gloeosporium perennans* in 3 weeks at 10° C. on apple agar of 15 different initial pH values from 2.0 to 11.8

A medium containing 4 per cent corn-meal agar and 0.5 per cent tannic acid was inoculated with mycelial tufts of *Neofabraea malicorticis* and *Gloeosporium perennans* and held at 10° C. for 21 days, and at 15° for 18 days. The results appear in Figure 4.

The tannic acid exerts an inhibiting influence on the growth of the two organisms, the effect being more marked for the *Neofabraea malicorticis*. With 0.5 per cent tannic acid the average retardation of

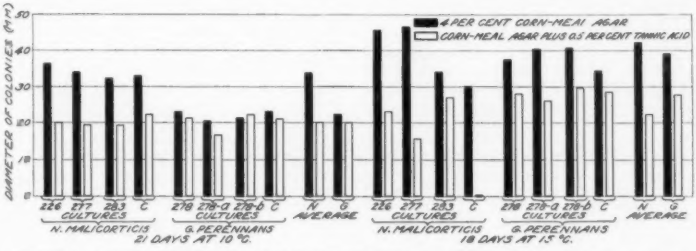
4 PER CENT CORN-MEAL AGAR
CORN-MEAL AGAR PLUS 0.5 PER CENT TANNIC ACID
DIAMETER OF COLONIES (MM)
50
40
30
20
10
0
226 277 283 C 278 278-a 278-b C N G 226 277 283 C 278 278-a 278-b C N G
CULTURES CULTURES CULTURES CULTURES
N. MALICORTICIS G. PERENNANS N. MALICORTICIS G. PERENNANS
AVERAGE AVERAGE
21 DAYS AT 10 °C. 18 DAYS AT 15 °C.

FIGURE 4.—Growth of four cultures each of *Neofabraea malicorticis* and *Gloeosporium perennans* on 4 per cent corn-meal agar and corn-meal agar plus 0.5 per cent tannic acid at 10° and 15° C.

growth for *N. malicorticis* at 10° C. was over three times as great as that for *Gloeosporium perennans*, and at 15° it was more than one and one-half times as great. As great differences were frequently found among strains of the same fungus as between the two fungi: For instance, at 15° C. the reaction of culture 283 of the anthracnose organism was more like that of the perennial canker than some of the cultures of Neofabraea (227 or Cooley's).

These studies were continued with the amount of tannic acid increased to 1 per cent. With this percentage the tannic acid usually

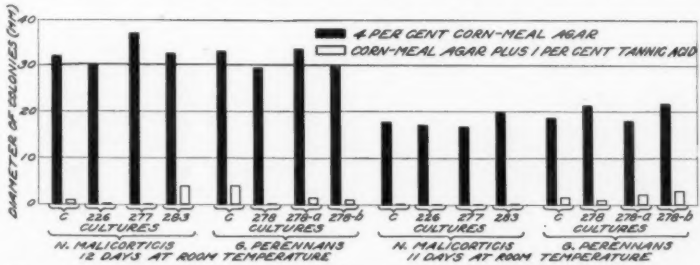
4 PER CENT CORN-MEAL AGAR
CORN-MEAL AGAR PLUS 1 PER CENT TANNIC ACID
DIAMETER OF COLONIES (MM)
40
30
20
10
0
C 226 277 283 C 278 278-a 278-b C 226 277 283 C 278 278-a 278-b
CULTURES CULTURES CULTURES CULTURES
N. MALICORTICIS G. PERENNANS N. MALICORTICIS G. PERENNANS
12 DAYS AT ROOM TEMPERATURE 11 DAYS AT ROOM TEMPERATURE

FIGURE 5.—Growth of four cultures each of *Neofabraea malicorticis* and *Gloeosporium perennans* on 4 per cent corn-meal agar and corn-meal agar plus 1 per cent tannic acid at room temperature

settles out after autoclaving, but if the medium is shaken while hot the tannic acid becomes dispersed again and remains so when the medium has become solid.

In 1 per cent tannic acid media the fungi grow with great difficulty at room temperature, there being no visible growth for a week or more. As will be seen from Figure 5, some of the strains had not begun growth at all at the end of 11 and 12 days, those that had started grew very feebly, and it was practically impossible to make any comparison between the effects on the two fungi.

TEMPERATURE STUDIES

Cultures of *Neofabraea malicorticis* and *Gloeosporium perennans* were grown on potato-dextrose agar at the temperatures 0°, 2.5°, 5°, 10°, 15°, and 20° C. The diameters of the colonies were measured at regular intervals. Duplicate readings were averaged and are presented in Figure 6. Within the range studied the increase in growth with increase in temperature was regular for both organisms, but the contrast in temperature responses between the two was no greater than between two cultures of the same organism.

STUDY OF APPLE ROTS

The same six temperatures were employed for a study of the apple rots produced by these two fungi. Temperature relations of apple-rotting fungi have been studied and reported by Brooks and Cooley (*2*). A number of common organisms attacking apple fruits were studied, among them *Neofabraea malicorticis*. Those writers state that they are not certain whether the culture was *N. malicorticis* or *Gloeosporium perennans*, as the latter had not yet been described but has since been shown to have existed at that time. However, the cultures were obtained from a part of the State of Washington where perennial canker rarely occurs, and in all probability the organism was *N. malicorticis*.

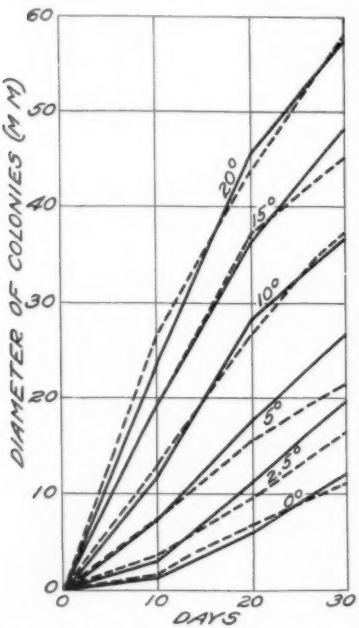

FIGURE 6.—Growth of *Gloeosporium perennans* (solid lines) and *Neofabraea malicorticis* (broken lines) on potato-dextrose agar at different temperatures

Apples possess several advantages over artificial media in the study of these two fungi. The fungi can not only be made to sporulate on the fruit, but they also produce macrospores more abundantly. On artificial media the microspores are produced in greater abundance. Jackson (*10*), writing of northwestern anthracnose, states that he frequently noticed the macrospores on apple fruits but was unable to obtain them by artificial inoculations except on the bark of young apple trees. In the present work apples could generally be relied on for the production of macrospores.

The apples were immersed in 50 per cent alcohol, then in mercuric chloride solution (1 to 1,000), and finally rinsed in hot water. Spores were inoculated into the sides of the apple fruit by means of a sterile needle. The apples were placed in moist chambers and held at the six temperatures heretofore mentioned. The diameters of the rots were measured once a week. The varieties used were Delicious, Grimes Golden, and Winesap. Ten apples were used for each organ-

ism at each temperature in all cases except for Grimes Golden apples, in which only two were used, and for the studies of Zeller's strains on Winesaps, in which only five were used. The results appear in Figures 7, 8, and 9. In Figure 7 the growth of *Gloeosporium perennans* is compared with that of *Glomerella cingulata* (Stoneman) Spaulding and Von Schrenk, the bitter-rot organism of the East.

The two fungi when grown on apples responded to the six temperatures in practically the same manner as they did when grown on artificial media. The growth curves for the two are similar. There was a gradual increase in growth with increase of temperature from 0° to 15° C. Between 15° and 20° the increase was not quite so rapid.

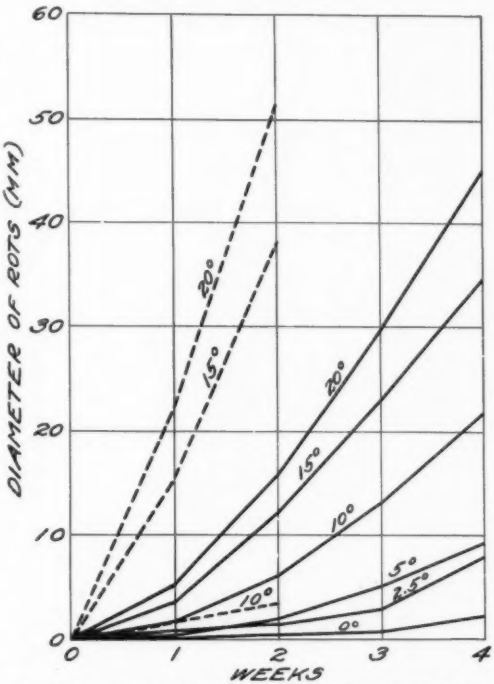

FIGURE 7.—Increase in diameter of rot on Grimes Golden apples produced by *Gloeosporium perennans* (solid lines) and *Glomerella cingulata* (broken lines) in 1 to 4 weeks at various temperatures

Zeller's three strains of *Gloeosporium perennans* and his three of *Neofabraea malicorticis* were inoculated into Winesap apples and held at the same six temperatures. The results appear in Figure 10. In this case different strains of the same organism grew at different rates, this being more marked with *N. malicorticis* than with *G. perennans*. Strain 283 of *N. malicorticis* made the most rapid growth, with 277 next, and 226 last. With *G. perennans*, strain 278 made the best growth, with 278–b next at temperatures of 5°, 15°, and 20° C. At temperatures 0°, 2.5°, and 10°, the two strains 278–a and 278–b showed little difference in rate of growth.

GERMINATION OF SPORES

For spore-germination studies apple fruits were inoculated with the two fungi and held at a low temperature until spores were produced. Distilled-water suspensions of the spores were then prepared in Van Tieghem cells and placed at temperatures 0°, 5°, 10°, 15°, 20°, 25°, and 30° C. The percentage of spores germinating, as well as the length of germ tubes, were determined at the end of 24 hours. At least four Van Tieghem cells were employed for each temperature,

and the experiment was repeated several times. In spite of the great number of spores counted there was considerable variation in percentage of germination, this being particularly true at 20°. The graphs in Figure 11 represent average percentage of germination based on the mass of evidence obtained.

Spores of both fungi failed to germinate at 0° C. within 24 hours, although they were found to have started at the end of 48 hours. Germination increased with rise of temperature from 0° to 15°, and the optimum appeared to be in the range of 15°, 20°, and 25°, with no great differences between any of these temperatures for either fungus. At 30° there was practically no germination for either fungus.

The increase in length of germ tube was consistent with increase of temperature up to 20° C. In Figure 12 it will be noted that the rate of growth declined at 25°. This applies to both organisms.

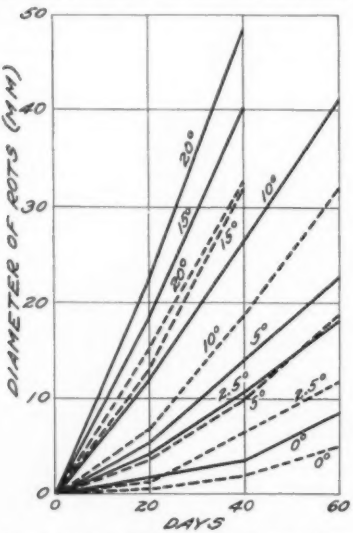

FIGURE 8.—Increase in diameter of rots on Delicious apples produced by *Gloeosporium perennans* (solid lines) and *Neofabraea malicorticis* (broken lines) in 20, 40, and 60 days at various temperatures

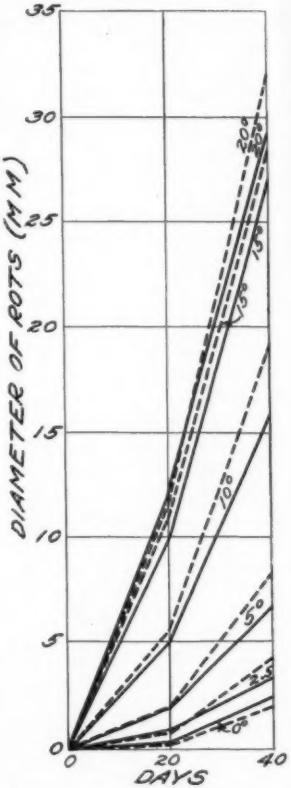

FIGURE 9.—Increase in diameter of rots on Winesap apples produced by *Gloeosporium perennans* (solid lines) and *Neofabraea malicorticis* (broken lines) in 20 and 40 days at various temperatures

SUMMARY AND CONCLUSIONS

Temperature responses of the two apple-rotting and canker fungi, *Neofabraea malicorticis* and *Gloeosporium perennans*, were very similar, whether inoculated into artificial media or into apple fruits. Both exhibited increased growth with rise of temperature in the range of 0° to 20°C., the rate of increase on apples falling off slightly from

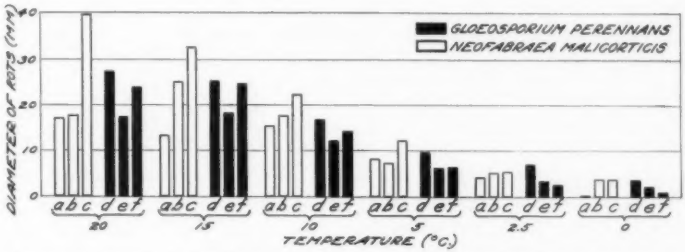

FIGURE 10.—Increase in diameter of rots on Winesap apples produced by three strains each of *Neofabraea malicorticis* and *Gloeosporium perennans* in 34 days at various temperatures. Neofabraea strains—*a*, 226; *b*, 277; *c*, 283. Gloeosporium strains—*d*, 278; *e*, 278-a; *f*, 278-b

15° to 20°. Both germinated and grew at 0° and rotted fruit at that temperature. Macrospores were readily obtained from inoculated apple fruits, being very abundantly produced at 10°.

In such nutritional studies as have been made, the two fungi have shown similar responses. The absence of potassium chloride or sodium nitrate from the nutrients in Dox's agar appeared to exert slight retarding effect on the growth of either organism. Gregory and Horne (*9*), working with other fungi, report that in the invasion of the apple fruit high resistance is associated with low water and low nitrogen content and high potash and high acidity. The present work is hardly comparable with that of Gregory and Horne, because their experiments concerned the apple fruit rather than artificial media, and they were studying different fungi.

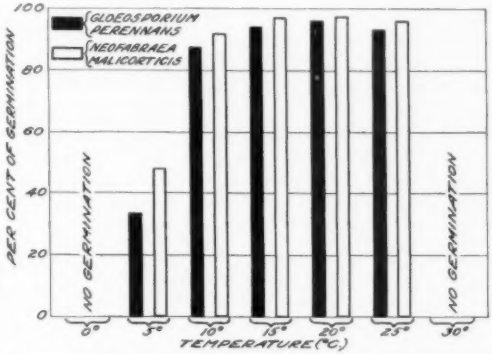

FIGURE 11.—Germination of *Gloeosporium perennans* and *Neofabraea malicorticis* in distilled water in 24 hours at various temperatures

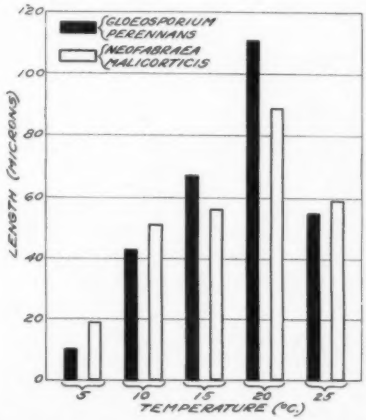

FIGURE 12.—Growth of germ tubes of *Gloeosporium perennans* and *Neofabraea malicorticis* in 24 hours at various temperatures

With regard to the hydrogen-ion studies, it is evident that both fungi experience difficulty in growing at a pH of less than 3.0 and can not grow at all at a pH of 2.0. In the alkaline range a pH of 11.8 produced definite inhibitory effects on both fungi.

Though the inhibition appeared slightly greater for *Gloeosporium perennans* than for *Neofabraea malicorticis*, germination studies indicate that there is no great difference in the responses of the two fungi in this range. It is realized that the organisms probably change the pH of the medium during growth and that the results indicate the ability of the fungi to make initial growth in the media of the various pH values. However, definite retardation of initial growth was noted in pH values of less than 3.0 and at 11.8, and pH values of 2.0 and 12.0 are the points where even initial growth can not be made.

The outstanding effect of the tannic acid medium is the retardation of growth of *Neofabraea malicorticis*. In some instances the fungus barely started growth by the end of 12 days; in others it was able to grow in the tannic acid medium, but the rate of growth was always retarded. From the results in Figure 4 it will be noted that the retardation is much more pronounced for Neofabraea than for Gloeosporium.

Optimum temperatures for germination of spores were found to be in the range of 15°, 20°, and 25° C. Though there was no germination at 0° in 24 hours, spores of both fungi were producing germ tubes in 48 hours, and a count revealed a germination of 34 to 46 per cent. At 30° there was no germination in 72 hours.

In the physiological studies conducted on *Neofabraea malicorticis* and *Gloeosporium perennans* there was greater variability among the different strains of the same species than between the two fungi themselves.

LITERATURE CITED

(1) Bavendamm, W.
1928. Über das vorkommen und den nachweis von oxydasen bei holzzerstorenden pilzen. Ztschr. Pflanzenkrank. u. Pflanzenschutz. 38:257–276, illus.

(2) Brooks, C., and Cooley, J. S.
1917. temperature relations of apple-rot fungi. Jour. Agr. Research 8:139–164, illus.

(2a) Brown, N. A.
1932. some pathological studies on apple cankers. Phytopathology 22 : 397–414, illus.

(3) Childs, L.
1927. perennial canker of apples. Oreg. State Hort. Soc. Ann. Rpt. 19:108–117.

(4) Clark, W. M.
1920. the determination of hydrogen ions; an elementary treatise on the hydrogen electrode, indicator and supplementary methods, with an indexed bibliography on applications. 317 p., illus. Baltimore.

(5) ——— and Lubs, H. A.
1916. hydrogen electrode potentials of phthalate, phosphate, and borate buffer mixtures. Jour. Biol. Chem. 25:479–510, illus.

(6) Cordley, A. B.
1900. apple tree anthracnose; a new fungous disease. Oreg. Expt. Sta. Bul. 60, 8 p., illus.

(7) Fisher, D. F.
1925. a new apple rot disease. ("bull's-eye rot"—caused by gloeosporium, zeller and childs). Wash. State Hort. Assoc. Proc. 21:44–48.

(8) ——— and Reeves, E. L.
1928. perennial canker. Wash. State Hort. Assoc. Proc. 24:55–61, illus.

(9) GREGORY, F. G., and HORNE, A. S.,
1928. A QUANTITATIVE STUDY OF THE COURSE OF FUNGAL INVASION OF THE APPLE FRUIT AND ITS BEARING ON THE NATURE OF DISEASE RESISTANCE. PART I. A STATISTICAL METHOD OF STUDYING FUNGAL INVASION. Roy. Soc. [London] Proc., Ser. B 102:427–466, illus.
(10) JACKSON, H. S.
1913. APPLE TREE ANTHRACNOSE. A PRELIMINARY REPORT. Oreg. Expt. Sta. Crop Pest and Hort. Rpt. 1911–12:178–197, illus.
(11) MCCRUMB, F. R., and KENNY, W. R.
1929. AN EXAMINATION OF POSSIBLE INDICATORS TO DETERMINE THE pH OF ALKALINE SOLUTIONS. Indus. and Engin. Chem., Analyt. Ed. 1:44–46.
(12) PECK, C. H.
1900. NEW SPECIES OF FUNGI. Bul. Torrey Bot. Club 27:14–21.
(13) ZELLER, S. M., and CHILDS, L.
1925. PERENNIAL CANKER OF APPLE TREES (A PRELIMINARY REPORT). Oreg. Agr. Expt. Sta. Bul. 217, 17 p., illus.

MACROCENTRUS ANCYLIVORUS ROH., AN IMPORTANT PARASITE OF THE ORIENTAL FRUIT MOTH[1]

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INTRODUCTION

An investigation of the oriental fruit moth (*Laspeyresia*) *Grapholitha molesta* (Busck), and its parasites, conducted at Riverton, N. J., in 1925 by Peterson and Haeussler (*4*)[3], showed that the very considerable reduction of the infestation by this pest in peach fruit was brought about chiefly by one species of parasite, a braconid of the genus Macrocentrus. Further studies by the writer (*3*), conducted in the same district from 1925 to 1928, showed that a great reduction in the population of the midsummer and late broods of twig-infesting larvae resulted each season from the severe parasitism by Macrocentrus in the larvae of the earlier broods. The average parasitism by this braconid in larvae collected from twigs ranged from 41 to 55 per cent for the four seasons. In 1925 and 1926, collections of larvae which were feeding in twigs during late June and early July showed parasitism by this one species as high as 92 and 84 per cent.

Cross parasitism and cross-breeding experiments by Stearns (*8*) and by the writer showed this parasite to be identical with that reared by Fink (*2*) from the strawberry leaf roller (*Ancylis comptana* Froel.) and described by Rohwer (*7*) as *Macrocentrus ancylivora*. The experiments consisted in the securing of parasitism of each host species by parasites reared from the other and the mating of female parasites from one host with males from the other, with subsequent propagation on both host species.

Because of the importance of this species as a parasite of the oriental fruit moth, it seemed advisable to make a detailed study of the species in its relation to this host. Its biology as a parasite of the strawberry leaf roller has been reported by Fink (*2*), and Stearns (*8*) published on its biology with reference to both *Grapholitha molesta* and *Ancylis comptana* as hosts. The present study deals entirely with the development of this parasite when bred from the oriental fruit moth.

Preliminary investigations on methods of handling the parasite under insectary conditions and a study of the habits of the species were begun in 1926. The more detailed study was taken up at Riverton in 1927, the insectary studies being started with the first adult parasites that emerged from the earliest parasitized fruit-moth larvae collected in the orchards that spring. In November, 1927, the insectary was moved from Riverton to Moorestown, N. J., a

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[3] Reference is made by number (italic) to Literature Cited, p. 100.

distance of about 5 miles, and the work was continued at Moorestown until the last hibernating individuals had emerged in the spring of 1929.

METHODS USED IN STUDYING THE PARASITE

The following methods were used in conducting the study of *Macrocentrus ancylivorus* under insectary conditions with *Grapholitha molesta* as a host: Grapholitha larvae, originating from eggs deposited upon pear leaves in the insectary, were allowed to begin their development by feeding in apples. When the larvae were from 4 to 8 mm long they were removed from the fruit as needed and placed on young, succulent peach twigs. The stems of the twigs were first inserted in a small bottle of water and held in a tight bunch by means of a cotton plug in the neck of the bottle. (Fig. 1.) A bunch of 8 or 10 twigs was found to accommodate from 10 to 15 larvae satisfactorily. Within an hour or two the larvae would spin a light silken web about their bodies to hold themselves against the twigs while eating their way in. The larvae were then in suitable position for being parasitized, and the bottle of twigs was placed in a cage containing parasites of both sexes. Late in the season, when young, succulent peach twigs were no longer available, good results were obtained by placing the partly grown larvae on cross-section slices of apple, about one-eighth inch thick. The larvae soon ate their way into the soft tissue where, owing to the thinness of the slices, the ovipositors of the female parasites could easily reach them when the slices were suspended on wire hooks in the parasite cages.

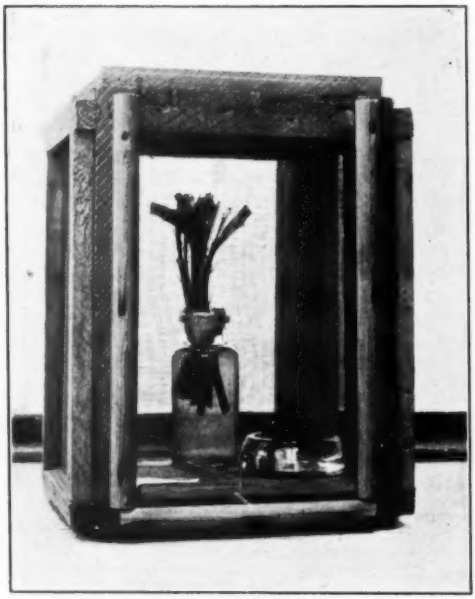

FIGURE 1.—Apparatus used for obtaining parasitism of larvae of *Grapholitha molesta*

The type of cage which proved most satisfactory for obtaining parasitism under insectary conditions is shown in Figure 1. This cage was 6 inches square and 8 inches high, with a solid wooden base and wooden framework covered on three sides and the top with 24-mesh galvanized wire screen. The entire fourth side was a sliding door of heavy transparent celluloid which could be raised for inserting or removing the bottle of twigs or the parasites. A glass caster cup containing a piece of sponge saturated with water was placed in the cage to provide moisture for the parasites. On extremely hot dry

days wet cheesecloth, draped over the top and the side of the cage opposite the door, aided materially in prolonging the life of the adult parasites. A high percentage of parasitism resulted when the twigs on which larvae were feeding remained in this cage from 4 to 24 hours, depending upon the number of female parasites present and the prevailing temperature. As a rule, the larvae were kept in the cage for 24 hours.

After the bottle of twigs was taken out of the cage, the twigs were removed and the lower parts of the stems which had been submerged in the water were cut off. The portions of twigs containing the larvae were placed in a jelly glass with one or more apples which provided additional food for the larvae when the twigs had become too dry. A narrow strip of corrugated paper was also placed in the glass, which was then covered with cheesecloth held tightly in place by rubber bands. (Fig. 2.)

FIGURE 2.—Jelly glass in which parasitized larvae continued feeding

When the larvae finished feeding and became full grown they left the fruit and spun their cocoons, usually in the corrugated paper. The glasses were examined once daily, and each corrugation containing a cocoon was cut out, given a record number, and placed in a small homeopathic vial until the adult emerged. Any full-grown larvae found crawling about the glass at the time of examination were placed in individual 2-dram vials stoppered with cloth-covered cotton plugs. These larvae spun their cocoons against the glass, and observations could readily be made of subsequent development without the necessity of disturbing them in any way. Each of these vials was examined once daily, and records were made for every individual whenever any important change occurred. Since it is usually only a matter of a few hours from the time the parasite larva emerges from the body of its host until it spins a cocoon of its own, the emergence of the parasite larva from its host was missed in many instances in the daily observations. Consequently, some of the vials were examined several times a day in order to determine the length of time that the larvae of Macrocentrus feed externally on the host. If a fruit-moth larva pupated it was known to be unparasitized, and subsequent records for that individual pertained to normal fruit-moth development.

For each individual reared to maturity in observation vials, the dates of moth egg deposition, parasite egg deposition, moth cocoon formation, parasite cocoon formation, pupation of unparasitized fruit

moths, and adult emergence of either parasite or moth were recorded. Thus it was possible to compute the length of the life cycle as well as the length of several of the developmental periods for each individual that emerged, whether Macrocentrus or Grapholitha.

THE ADULT

DESCRIPTION

The following description of the adult of *Macrocentrus ancylivorus* (fig. 3) is largely a condensation of the original description by Rohwer (*7*) and subsequent descriptions by Fink (*2*) and Stearns (*8*).

FIGURE 3.—Adults of *Macrocentrus ancylivorus*: A, Female; B, male. × 4.5

The female averages about 4.5 mm in length, the ovipositor 5.5 mm, antenna 7.5 mm. and wing expanse about 9 mm, male often slightly smaller; General body color pale ferruginous; eyes and ocelli black; antennae uniform dark brown distad of the pedicel, segmentation not clearly distinct except with a strong lens; base and apex of abdomen of both sexes sometimes marked with dark brown; face, ventral part of antennal scape and pedicel, ventral part of thorax, and sometimes venter of abdomen lighter colored, almost yellow; legs yellow.

SIMILARITY TO MACROCENTRUS DELICATUS CRESS.

In 1926, a few lighter colored adults of Macrocentrus were noted among those emerging from field-collected material. At first these were considered as merely color variations of the one species, but all such individuals were noted in the records, and the specimens were preserved. Similar adults were obtained in 1927, and, since these emerged each season from larvae that had been collected over a period of about two weeks during late June and early July, it was suspected that they might be of a separate species. Mating was never observed between light-colored individuals of one sex and dark-colored individuals of the opposite sex, whereas both light and dark individuals mated readily with similar adults of the opposite sex. It was further noted in 1927 that dark-colored females which had been fertilized always produced dark-colored progeny and that fertile light-colored females always produced light-colored progeny. R. A. Cushman, of the taxonomic unit of the Bureau of Entomology, later determined the light-colored specimens to be a distinct species, *M. delicatus* Cress.

Morphological differences occurring in the two species, as well as characters useful in distinguishing adults of *ancylivorus* from those of *delicatus*, have been very fully discussed by Driggers and Pepper (*1*). The writer found the difference in appearance of the antennae and the difference in general body color to be the most useful characters for distinguishing living adults of one species from those of the other species. That the antennae of *delicatus* are longer and have a segmented or "beaded" appearance whereas those of *ancylivorus* are shorter and are "hairlike" in appearance, as mentioned by the authors cited, was noted by the writer in 1927 and used at that time as a distinguishing character.

MATING

When adults of both sexes of *Macrocentrus ancylivorus* are confined together the male usually attempts mating almost immediately. The wings of the male are raised almost perpendicularly and flutter rapidly as he persistently follows the female. Upon approaching close to the female from the rear the male curls the tip of the abdomen forward from beneath in an endeavor to reach the female. The latter often flies out of reach, and it is sometimes necessary for the male to make several attempts before mating is accomplished.

For 18 observed matings the minimum, average, and maximum duration of the act was 12, 22.5, and 45 seconds, respectively. After mating, the female usually repels further attempts on the part of the same or other males, although in one instance when one female was confined in a 6 by 8 inch glass jar with three males the female was observed mating four distinct times for 30, 12, 19, and 25 seconds, with intervals of 4, 1½, and 11 minutes, respectively. The last three matings were with the same male. A male may mate with several females; thus when more females than males are placed in a cage most of the females usually become fertilized. Mating activity is most noticeable in the cages on warm summer days and during the warmer hours of the day in spring and fall.

PROPORTION OF SEXES PRODUCED

More females than males of *Macrocentus ancylivorus* are recovered from larvae of the oriental fruit moth parasitized normally in the orchards in the vicinity of Riverton, N. J. For the three consecutive seasons, 1926, 1927, and 1928, the percentage of female parasites which emerged from parasitized larvae collected in infested peach twigs was consistently greater than the percentage of males. During the respective seasons, 55.2, 55.5, and 53.4 per cent of the parasites produced were females. A total of 2,642 adult parasites were reared from fruit-moth larvae parasitized in the field over the 3-year period. Of these, 1,443, or 54.6 per cent, were females and 1,199, or 45.4 per cent, were males.

A greater proportion of female parasites was also obtained from larvae of the strawberry leaf roller parasitized in the field in the same region. Collections of larvae of this host species in 1927 and 1928 produced 58.1 and 58.8 per cent female parasites during the respective seasons. From the total of 219 parasites that emerged during the two years, 128, or 58.4 per cent, were females and 91, or 41.6 per cent, were males.

A consistently greater proportion of females, such as occurs under natural conditions, is not always produced when this species is bred under insectary conditions. Of the total number of parasites reared from Grapholitha in the insectary in 1927 in connection with the life-history studies, 64.4 per cent were females. In similar rearings in 1928 only 48.8 per cent were females. However, from the total of 1,562 parasites bred from this host under insectary conditions during the two seasons 894, or 57.2 per cent, were females and 668, or 42.8 per cent, were males.

For securing parasitism of larvae in the insectary, parasites of both sexes were always placed in the oviposition cages. However, it is quite possible that in some instances parasitism occurred before all of the female parasites had been fertilized. Very probably this happened on several occasions in 1928 when, owing to a shortage of males, more female than male parasites were placed in the oviposition cages. This may explain, in part at least, the high percentage of male parasites that was produced during that season, since unfertilized eggs of *Macrocentrus ancylivorus* yield males only.

LONGEVITY

Twenty-two separate experiments were conducted during the season of 1927 to determine the length of adult life when parasites of both sexes were confined together in cages such as those previously described, with either fruit-moth larvae or larvae of the strawberry leaf roller. Records were obtained from a total of 115 female and 79 male parasites. The minimum, average, and maximum length of life for both sexes confined together was 1 day, 6.7 days, and 14 days, respectively. The highest average longevity for the females in any single experiment was 11.1 days, for the males 11.3 days, and for the two sexes together 10.6 days.

For eight unfertilized females that were confined in a cage without males, but provided with Grapholitha larvae in which to oviposit, and fed a 10 per cent solution of honey water, the minimum, average, and maximum length of life was 13, 17.9, and 29 days. When freshly emerged males were confined in a cage with no females and no larvae present, but fed the 10 per cent solution of honey water, the respective duration of life was 7, 10.3, and 19 days.

OVIPOSITION

Oviposition by *Macrocentrus ancylivorus* on *Grapholitha molesta* is most readily accomplished when the larva of the host species is in a position from which it can not easily escape the thrusts of the ovipositor of the female parasite. The most favorable position for attack seems to be when the larva is feeding within a peach twig or a thin slice of apple, or is inclosed in a light feeding web preparatory to entering a twig. Successful oviposition has never been observed by the writer when the host larva was not in some such fixed position. If free to escape, the larva wriggles out of reach at the first touch of the ovipositor and thus frightens the parasite.

When a number of full-grown larvae of the oriental fruit moth were killed in hot water, inserted in nail punctures in a piece of dried cornstalk, and placed in an oviposition cage, female parasites oviposited readily in the bodies of the dead larvae. The dead hosts dried up,

however, before the parasite eggs could develop. On several occasions females were observed endeavoring to parasitize full-grown Grapholitha larvae that were wandering about the cage or other container, but the larvae always managed to escape the thrust of the ovipositor, and in no instance was such attempted parasitization successful.

Females of this species have been observed ovipositing at practically all hours of the day during warm summer weather. On several occasions, when a flash light was suddenly turned on the oviposition cages after dark, parasites were observed ovipositing as late as 10 p. m.

Under insectary conditions a female parasite will oviposit several times in the same larva. One female was observed to puncture a larva 17 times and another female oviposited 13 times in a single larva. Dissections of 12 hibernating Grapholitha larvae which had been parasitized in cages in the insectary showed that, although superparasitism occurs, only one parasite larva survives regardless of the number of eggs deposited. Each host larva contained one living parasite larva. In 11 of the host larvae from 1 to 58, or a total of 131, head capsules and bodies of dead parasite larvae were also present. The remaining host larva contained no sign of superparasitism.

PARTHENOGENESIS

Unfertilized females can deposit eggs that will mature, but the progeny are always males. In 1927 a cage containing 10 freshly emerged unfertilized females was started on July 28. From numerous insectary-bred larvae of the oriental fruit moth which were placed in this cage a few at a time, a total of 63 parasites was produced, all of them males. When some of these males were allowed to mate with unfertilized females emerging from larvae of the same host that had been parasitized in the field, normal progeny of both sexes were produced.

STAGE OF HOST ATTACKED

Macrocentrus ancylivorus will attack the feeding larvae of the oriental fruit moth in any stage of development, from the very small first-stage larvae to those that are nearly full grown. Table 1 shows the parasitism by this species which occurred in Grapholitha larvae of various sizes taken in weekly collections of infested twigs from orchards near Riverton during the season of 1927. Each larva was removed from the twig within an hour after it had been collected, and its length in millimeters was recorded. The larvae were divided into three groups, according to length, and placed on apples in jelly glasses to continue their development. One group contained larvae measuring from 1.5 mm (recently hatched) to 3.5 mm, inclusive, another group contained those measuring over 3.5 to 6.5 mm, inclusive, and the third group contained those measuring over 6.5 mm. Practically all the larvae of the third group were in the last larval instar. The table shows, for each respective size group, the number of Grapholitha larvae collected, the total number of individuals that completed development and emerged as adults (including moths and all species of parasites), and the number of adult *M. ancylivorus* that emerged. The percentage of all individuals that reached maturity, based on the number of larvae collected, and the percentage of *M. ancylivorus* that emerged, based on the total emergence, are also given for each of

the three groups and for the combined total. It will be noted that the percentage of individuals reaching maturity increased decidedly with each increase in the size of the larvae when collected. This is explained, in part at least, by the fact that more of the smaller larvae were injured in handling and failed to enter the new food medium after having been removed from the twigs. There was also an increase in the percentage of Macrocentrus that emerged from the larvae of each progressive size.

TABLE 1.—*Numbers and percentages of individuals reared to maturity and Macrocentrus ancylivorus adults emerged from Grapholitha molesta larvae of various sizes taken in field collections of infested peach twigs at Riverton, N. J., 1927*

Grapholitha larvae		Adults, both moths and all parasites, emerging		Macrocentrus ancylivorus emerging	
Size when collected	Collected				
	Number	*Number*	*Per cent*	*Number*	*Per cent*[a]
1.5 to 3.5 mm	900	193	21.44	97	50.26
Over 3.5 to 6.5 mm	974	361	37.06	193	53.46
Over 6.5 mm	1,015	651	64.14	379	58.22
Total or average	2,889	1,205	41.71	669	55.52

[a] Based on total emergence of moths and parasites.

Under insectary conditions it was also found that the percentage of individuals reaching maturity was greater with each increase in the size of the larvae at the time these were transferred from apples to twigs before being placed in the parasite oviposition cages. The majority of the larvae used in the life-history studies ranged in length from 4 to 8 mm, inclusive. During both 1927 and 1928, fruit-moth larvae ranging from 4 to 6.5 mm in length were more heavily parasitized than those measuring 7 mm and over. The percentages of parasites that emerged from a considerable number of larvae that had been separated into these two size groups were 56.98 and 47.96, respectively, in 1927, and 83.33 and 70.81, respectively, in 1928. There were not sufficient numbers of larvae measuring from 1 to 3.5 mm used in the insectary studies to provide comparative data on the percentage of parasitism occurring in these smaller larvae. Of the total number of adults reared from all larvae subjected to the attack of Macrocentrus in the insectary in 1927, 1928, and in both seasons combined, 54.72, 71.10, and 61.23 per cent, respectively, were parasites.

Parasitism of *Grapholitha molesta* in the field is confined largely to those larvae of the first, second, and a portion of the third broods which feed within the peach twigs, for when larvae of the late broods enter the fruit it is difficult for the female Macrocentrus to reach them with her ovipositor. In 1925 a large number of larvae of the oriental fruit moth were removed from Elberta peaches at harvest time. From these larvae, 627 moths and only 1 *M. ancylivorus* emerged. The parasitism by this species in larvae that had infested the twigs in that same orchard earlier in the season was very high.

This parasite does not attempt to attack full-grown Grapholitha larvae that have formed cocoons, nor does it attempt to oviposit in naked Grapholitha pupae.

REPRODUCTIVE CAPACITY

Four females of *Macrocentrus ancylivorus* were mated within a few hours after they had emerged and were then placed in cages, only one parasite to a cage, and each was provided with 50 larvae of the oriental fruit moth feeding on bundles of twigs. A new lot of 50 larvae was placed in each cage every second day until the parasite died. The four females lived 8, 4, 5, and 6 days; and the number of progeny produced by each individual was 40, 28, 29, and 22, respectively, 119 in all, or an average of 29.75 each.

LIFE HISTORY AND COMPARATIVE RATE OF DEVELOPMENT OF THE PARASITE AND THE HOST

The writer had planned to make a detailed study during the season of 1929 of that period in the development of *Macrocentrus ancylivorus* during which the parasite lives within the body of the host larva. Circumstances, however, prevented continuation of investigations with this parasite after the spring of 1929. Consequently, information is not available regarding the duration of the egg stage and the number and duration of the larval instars. Data concerning the minimum, average, and maximum number of days required for the several periods of development and for the complete life cycle of *M. ancylivorus* and its host *Grapholitha molesta* during 1927 and 1928 and for the two seasons combined are presented in Table 2. These data are recorded separately for transforming [4] and for wintering [4] individuals. In each instance the number of parasites or fruit moths from which observations were made is indicated.

[4] The terms "transforming" and "wintering" are used here in the same sense in which they were used by Peterson and Haeussler (6) in their life-history study of the host species. "Transforming" refers to those individuals which, completing their life cycle, transform to adults the same season the eggs are deposited; "wintering" refers to those individuals which hibernate and do not become adults until the following season.

TABLE 2.—*Average, minimum, and maximum number of days required for several periods of development and for the life cycle of Macrocentrus ancylivorus and Grapholitha molesta in 1927 and 1928, and the number of individuals on which data are based, Riverton and Moorestown, N. J.*

MACROCENTRUS ANCYLIVORUS

Period of development	Time required for development										Individuals observed					
	Transforming					Wintering					Transforming			Wintering		
	1927	1928	Two years combined			1927	1928	Two years combined			1927	1928	Two years combined	1927	1928	Two years combined
	Average	Average	Average	Minimum	Maximum	Average	Average	Average	Minimum	Maximum						
Parasite egg deposition to formation of host cocoon	*Days* 8.3	*Days* 10.5	*Days* 9.4	*Days* 2	*Days* 31	*Days* 12.2	*Days* 25.9	*Days* 16.7	*Days* 7	*Days* 44	*Number* 287	*Number* 313	*Number* 600	*Number* 33	*Number* 16	*Number* 49
Host cocoon formation to emergence of parasite larva	7.8	6.0	6.9	1	23	200.5	169.2	190.31	141	212	122	126	248	33	16	49
Emergence of parasite larva to formation of its cocoon	.6	.5	.5	[a].5	4	2.4	1.4	2.1	[a]0.5–1	6	122	126	248	33	16	49
Formation of cocoon to emergence of adult	16.4	14.0	15.2	9	69	37.6	40.5	38.5	30	52	287	313	600	33	16	49
Life cycle [b] (observation vials only)	33.1	31.1	32.1	21	91	252.7	237.1	247.6	224	262	287	313	600	33	16	49
Life cycle (including cocoons in corrugated paper)	32.9	29.7	31.2	20	91	243.4	243.1	243.3	216	280	582	653	1,235	259	68	327
GRAPHOLITHA MOLESTA																
Egg deposition to formation of cocoon	22.0	28.2	24.6	14	51	31.8	42.4	35	13	89	139	99	238	66	28	94
Formation of cocoon to pupation	3.9	3.9	3.9	1	13	210.6	184.4	202.8	108	256	139	99	238	66	28	94
Pupation to emergence of adult	11.6	9.3	10.6	7	15	31	34.3	32	9	40	139	99	238	66	28	94
Life cycle (observation vials only)	37.5	41.4	39.2	26	61	273.3	261.1	269.7	236	308	139	99	238	66	28	94
Life cycle (including cocoons in corrugated paper)	35.7	37.9	36.3	23	62	275.2	272.6	274.3	236	337	479	173	652	217	120	337

[a] Approximation.
[b] "Life cycle" includes the complete development, from deposition of the egg to emergence of the adult.

DEVELOPMENT OF THE PARASITE LARVA WITHIN THE HOST

The parasite larva which hatches from the egg deposited within the body of the partly grown Grapholitha larva spends the greater portion of its larval life as an internal parasite. When the Grapholitha larva has finished feeding in its last stage and reached the quiescent period within its cocoon the parasite larva, having attained its last instar, makes its way out of the host. During the two seasons of 1927 and 1928 (combined), unparasitized transforming Grapholitha required from 14 to 51 days, or an average of 24.6 days, from the time the egg was deposited until the cocoon was spun; and unparasitized wintering Grapholitha required from 13 to 89 days, or an average of 35 days. Parasite eggs which produced parasites transforming during the same season were deposited in the host larvae from 2 to 31 days, or an average of 9.4 days, before the formation of the host cocoon; and those producing wintering parasites were deposited from 7 to 44 days, or an average of 16.7 days, before the formation of the Grapholitha cocoon.

Transforming fruit-moth larvae, when unparasitized, pupate in from 1 to 13 days, or an average of 3.9 days after the cocoon has been spun. The transforming parasite larva emerges from the body of the Grapholitha larva in from 1 to 23 days, or an average of 6.9 days, after the host larva has constructed its cocoon. Therefore, the transforming parasite larva emerges from the body of the host on an average of 3 days after the fruit-moth larva would normally have pupated.

FIGURE 4.—Hibernating larva of *Macrocentrus ancylivorus*. × 12.5

Wintering parasite larvae remain within the body of the hibernating host larvae throughout the winter. The hibernating larva of *Macrocentrus ancylivorus* (fig. 4) averages 2.63 mm in length and 0.43 mm in width. The heavily sclerotized, light-brown head measures 0.24 mm in length by 0.27 mm in width and bears a pair of very strong, heavily sclerotized mandibles. The body, which is of a dirty yellowish white, consists of 13 segments besides the head and is terminated by three hooklike prolongations of the anal segment. The hibernating larvae coincide in all respects with the description given by Fink (*2*) of the mature first-instar larva of this species.

In the spring, the hibernating larvae attain their last larval instar as internal feeders and then come out of the body of the host, the first parasite larvae appearing externally a few days after the first unparasitized fruit-moth larvae have pupated. Parasite eggs which produce hibernating parasites are deposited later in the fall than are Grapholitha eggs which produce hibernating individuals. The average length of time from formation of the host cocoon in the fall to emergence of the parasite larva the following spring (190.3 days) is considerably shorter than the period from the formation of the cocoon to the transformation to pupa (202.8 days) for unparasitized wintering individuals of the host species. Fruit-moth larvae that are parasitized by *M. ancylivorus* do not pupate, as the host larva is always destroyed previous to pupation.

DESTRUCTION OF THE HOST

When the parasite larva comes out of the host it lies parallel to the body of the host larva with its head embedded in the hole from which it emerged. It then proceeds to feed on its former host until nothing remains but the sclerotized parts and the shriveled skin. During warm summer weather the average transforming parasite larva finishes feeding in from about 12 to 18 hours after emerging from its host. Wintering parasites, the larvae of which emerge from the host during cool spring weather, require an average of about 2 days to complete their feeding. While feeding externally, the dirty yellowish-white parasite larva shows up distinctly against the darker grayish pink of the host larva. (Fig. 5.) It increases rapidly in size as it feeds, and when full grown is from one-half to two-thirds the size of its former host, averaging about 5 to 6 mm in length.

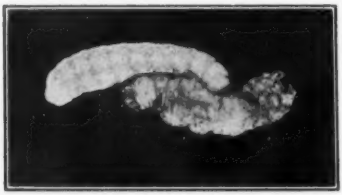

FIGURE 5.—Partly grown Macrocentrus larva feeding on a larva of the oriental fruit moth. × 5

FORMATION OF THE PARASITE COCOON

Shortly after the full-grown Macrocentrus larva has finished feeding, construction of the parasite cocoon begins. This cocoon is constructed within the old cocoon of the former host. The shriveled skin, sclerotized head, and thoracic and anal shields of the host larva also remain within the host cocoon. At first a web of very fine, white, silky threads appears about the parasite larva. (Fig. 6, A.) As construction continues the cocoon gradually becomes light brown in color. The completed cocoon (fig. 6, B), which makes a very strong protection about the prepupa and pupa, is dark amber in color and oval in shape and averages from 5.5 to 6.5 mm in length by 1.5 to 2 mm in width. About 24 hours are required for the spinning of the cocoon. The adult emerges from the cocoon by making a slit which opens the round, somewhat thickened anterior end. (Fig. 7.)

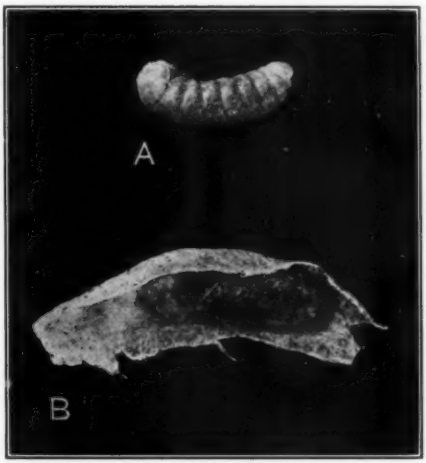

FIGURE 6.—Cocoon of *Macrocentrus ancylivorus:* A, Full-grown larva beginning to construct cocoon; B, completed cocoon shown within a cocoon of the oriental fruit moth. × 4.5

PREPUPAL AND PUPAL PERIODS

The time required from formation of the parasite cocoon to emergence of the adult, including the prepupal and pupal periods, averaged 15.2 days for transforming parasites (Table 2), with a minimum of 9 days and a maximum of 69 days. For wintering parasites, the period of time within the parasite cocoon averaged considerably longer, 38.5 days, with a minimum of 30 days and a maximum of 52 days. This was due to the fact that the cocoons of hibernating parasites were all spun early in the spring when the weather was cool, and thus the prepupal and pupal periods were of longer duration.

The time spent within the cocoon by unparasitized Grapholitha has been separated into two periods, prepupal and pupal. (Table 2.) The former period, indicated as "formation of cocoon to pupation" averaged 3.9 days for transforming moths and 202.8 days for wintering moths. The remainder of the time within the cocoon, "pupation to emergence of adult," required an average of 10.6 days for transforming Grapholitha and 32 days for wintering ones, the minimum and maximum time for this period being, respectively, 7 and 15 days for transforming individuals, and 9 and 40 days for wintering individuals.

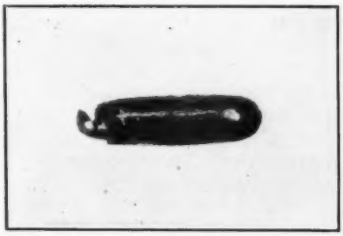

FIGURE 7.—Macrocentrus cocoon from which the adult has emerged, showing opening at the anterior end. × 4.5

LIFE CYCLE OF PARASITE AND HOST

The life cycle of *Macrocentrus ancylivorus*, from deposition of the egg to emergence of the adult, averaged 32.1 days for the 600 transforming parasites and 247.6 days for the 49 wintering parasites in observation vials during the two seasons. Records on the life cycle were also available from parasites the host cocoons of which had been spun in corrugated paper. When the two sets of records were combined, the life cycles of the 1,235 transforming parasites were found to average 31.2 days, the minimum and maximum life cycles being 20 days and 91 days, respectively. The minimum, average, and maximum life cycles of the 327 wintering parasites in the combined records were 216, 243.3, and 280 days, respectively.

For unparasitized fruit moths reared in observation vials during the two seasons the average life cycles were 39.2 days for 238 transforming moths and 269.7 days for 94 wintering moths. When records of those individuals that had spun cocoons in corrugated paper were included, the minimum, average, and maximum life cycles of 652 transforming moths were 23, 36.3, and 62 days, respectively, and the corresponding life cycles of 337 wintering moths were 236, 274.3, and 337 days, respectively.

The life cycle of the parasite is in each instance considerably shorter than that of the host species. The average life cycle of all transforming parasites was 5.1 days shorter than that of all transforming moths, and for all wintering parasites 31 days shorter than for all wintering moths.

AVERAGE LIFE CYCLE AND RELATION TO TEMPERATURE

The average number of days required for the life cycle of transforming parasites and moths maturing from eggs deposited on each day throughout the seasons of 1927 and 1928 is shown in Figures 8 and 9. The average life cycles of all transforming Macrocentrus that emerged as adults throughout the season are indicated by the height of the solid day line above the base line on the date of oviposition. The average life cycles of transforming Grapholitha that emerged as adults throughout the season are similarly shown by the height of the dotted day line above the base line on each oviposition date.

The relation between prevailing temperature and the length of the life cycle in each case is also shown. The height of the solid and dotted temperature lines for each date indicates the average of all temperatures which existed during the average life-cycle periods of all individuals that emerged as adults from eggs deposited on that date, for parasites and moths, respectively. For example, the average life cycle of all transforming Macrocentrus, the eggs of which were deposited on June 27, 1927, was 26 days; and the average temperature recorded for that period, as indicated by the solid temperature line on that date, was 72.8° F. This is the average of all temperatures for 26 days, starting with June 27, based on thermograph readings at 2-hour intervals throughout the entire period. The average life cycle of all transforming Grapholitha, the eggs of which were deposited on that same date, was 32.3 days; and the average temperature recorded for that period, as indicated by the dotted temperature line on June 27, was 73.2° F. This is the average of all temperatures for 32.3 days starting with June 27.

SPRING EMERGENCE OF HIBERNATING PARASITES AND MOTHS

It has previously been pointed out by the writer (*3*) that, in the vicinity of Riverton, N. J., adults of *Macrocentrus ancylivorus* emerge sufficiently early in the spring under field conditions to attack the first fruit-moth larvae which enter twigs.

During the winter of 1927–28 Grapholitha larvae which had been subjected to parasitism by Macrocentrus in the insectary during the fall of 1927 were allowed to hibernate outdoors exposed to natural conditions of temperature and weather. The larvae had constructed cocoons in narrow strips of corrugated straw paper which were firmly tacked in open screen cages, identical with one of the types of cages (fig. 10) employed by Peterson and Haeussler (*5*) in conducting spring-brood emergence studies of the oriental fruit moth. Two cages, each containing approximately 230 cocoons, were hung on a post. One cage was placed on the south side of the post, as close to the ground as possible, and the other was placed on the north side of the post, about 5 feet above the ground. Previous experiments with hibernating oriental fruit moths and codling moths had shown that the larvae in cages placed in these two positions were subjected to the two extremes of temperature and sunlight during the spring to which larvae normally hibernating on the trunks of trees in the orchard are exposed, and that, consequently, the earliest and latest emergence of adults in the spring occurs in the south-low and north-high cages, respectively.

As had been expected, the first emergence of parasites in the spring of 1928 occurred in the south-low cage, and, as a matter of fact, all

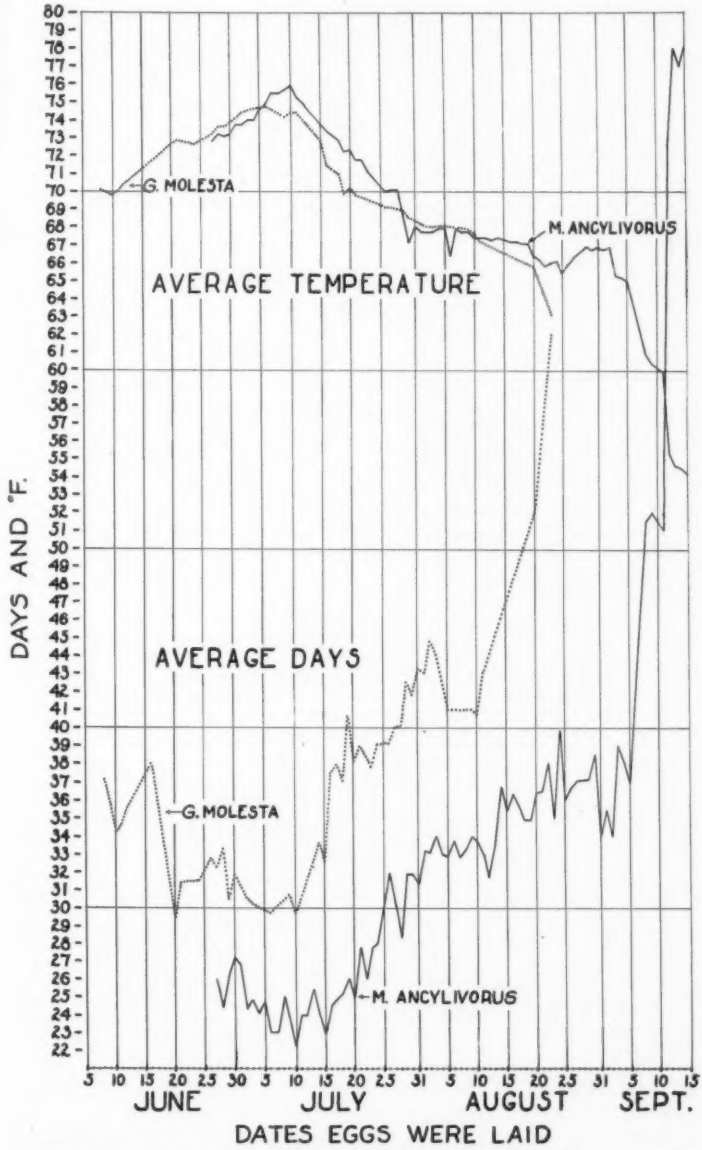

FIGURE 8.—Comparative average life cycles of transforming individuals of *Macrocentrus ancylivoras* (solid line) and *Grapholitha molesta* (dotted line) with corresponding average temperatures in 1927. The life cycle is indicated by the height above the base line (at 0, not shown) on the date on which the eggs were laid. The temperature indicated for that date is the average temperature for the entire life cycle that began on that date

the parasites in this cage had emerged two days before emergence began in the north-high cage. The first adult Macrocentrus appeared in the south-low cage on May 11, the peak of emergence (that is, the date on which 50 per cent had emerged) occurred on May 17, and the last parasite emerged on May 19. In the north-high cage, first emergence was on May 21, the peak of emergence was reached on May 24, and the last emergence occurred on June 12.

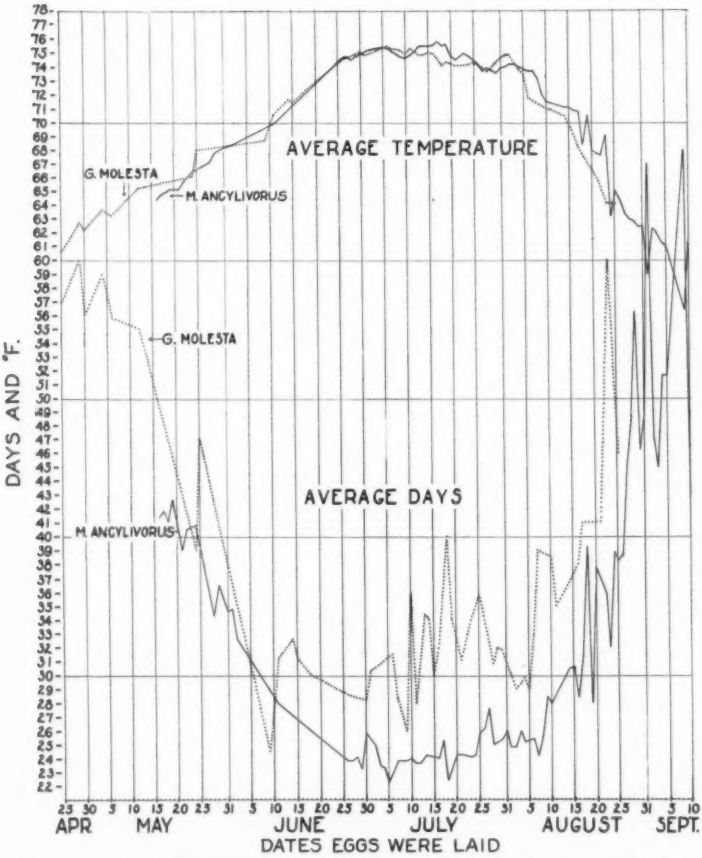

FIGURE 9.—Comparative average life cycles of transforming individuals of *Macrocentrus ancylivorus* (solid line) and *Grapholitha molesta* (dotted line) with corresponding average temperatures in 1928. The life cycles and temperatures are indicated as explained in the legend of Figure 8 and in the text

Two similar cages containing unparasitized larvae of the host species had been placed in corresponding positions on a near-by pole. The first adult moth emerged in the south-low cage on April 8, the peak of emergence in this cage was reached on April 30, and last emergence occurred on May 7. In the north-high cage the first moth

emerged on May 8, the peak of emergence occurred on May 16, and the last moth emerged on June 12.

By combining the emergence data from the south-low and north-high cages, in the case of both the parasite and the moth, a general idea is obtained of the spring emergence of the two insects closely approximating that which normally occurs in the peach orchards, assuming that hibernating cocoons under natural conditions are more or less evenly distributed in these two extreme and all intermediate locations.[5]

FIGURE 10.—Type of outdoor hibernation cage from which the spring-emergence records were obtained for both parasites (*Macrocentrus ancylivorus*) and moths (*Grapholitha molesta*)

The comparative emergence of the parasite and the host species in the outdoor cages and in the insectary during the spring of 1928 is shown in Figure 11. The solid lines represent parasite emergence and the broken lines emergence of the moth. The dates of first and last emergence are indicated on the base line and the peak of the triangle denotes the date on which the peak of emergence was reached in each instance.

It will be noted that in the outdoor cages the first moth emerged on April 8, more than a month earlier than the emergence of the first parasite on May 11. Of both species, the earliest individuals to emerge were males, the first female moth emerging on April 20, which was 25 days before the emergence of the first female parasite on May 15. The peak of moth emergence was reached on May 4, which was 19 days before the peak of parasite emergence on May 23. The last individuals of both parasite and host species emerged on June 12.

Spring emergence was considerably delayed in the case of hibernating cocoons which had been kept in the insectary throughout the winter and spring. As shown in Figure 11, the first moth from this material emerged on May 10, the peak of emergence was reached on May 30, and the last moth emerged on July 1. This represents a delay of 32, 26, and 19 days, respectively, as compared to moth

[5] A closer approximation of normal orchard emergence would undoubtedly have been obtained by the use of eight cages, four adjacent to the ground and four 5 feet above the ground, with one cage of each group facing north, east, south, and west. Data regarding the emergence of the host species were available for the entire eight cages but since sufficient hibernating parasite material was available for only two cages, the emergence data for the moths, as given here, were taken from only the two corresponding cages of hibernating Grapholitha material.

emergence in the outdoor cages. In the case of the parasites the delay was not so great. Nevertheless a retardation of emergence occurred, the first adult (a female) appearing on May 19, 8 days later than the first parasite emerged in the outdoor cages. The peak of emergence was delayed 4 days, being reached on May 27; and the last parasite emerged on June 13, only 1 day after the last individual emerged in the outdoor cages. Thus it is apparent that, in the case of the parasites as well as the moths, a closer approximation to the normal spring emergence can be obtained by the use of outdoor hibernation cages. In the insectary, the first parasite emerged 9 days later than the first moth. The peak of parasite emergence, however, was reached 3 days in advance of the peak of moth emergence, and the last moth emerged 18 days later than the last parasite.

SEASONAL EGG DEPOSITION

A comparison of the periods during which fruit-moth eggs and parasite eggs were deposited in 1927 and 1928, from which transforming and wintering individuals of Grapholitha and Macrocentrus

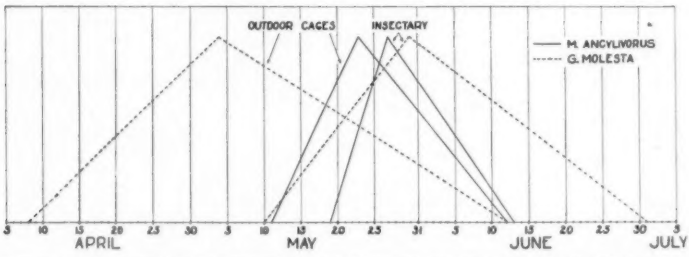

FIGURE 11.—Comparative emergence of adults of *Macrocentrus ancylivorus* and *Grapholitha molesta* during the spring of 1928 from larvae that hibernated in outdoor cages and from larvae that hibernated in the insectary

subsequently emerged, is presented in Figure 12. The dates on which the first and last eggs producing transforming and wintering individuals were deposited are indicated in each instance by the range of the horizontal lines, the broken and solid lines representing the fruit-moth eggs and parasite eggs, respectively.

In 1927, the first Grapholitha eggs of the season were deposited in the insectary on April 23 by females which emerged in outdoor cages, but the first eggs on which developmental records were kept in connection with the parasite studies were deposited on June 8. The last eggs that produced transforming moths were deposited on August 23. The first eggs producing wintering Grapholitha were deposited on July 27. Thus both transforming and wintering fruit moths developed from eggs deposited between July 27 and August 23, inclusive. The last eggs that produced wintering Grapholitha, which were also the last eggs of this species laid that season, were deposited on September 24.

Since it was not possible to begin the parasite life-history studies in 1927 until adults of the first brood began to emerge from parasitized host larvae collected in the field, the exact date on which the first parasite eggs of that season were deposited is not known. However, the first Grapholitha larvae found in peach twigs in the orchard that

season, which were found on May 11, were parasitized by *Macrocentrus ancylivorus*. Therefore, it is certain that female parasites had emerged from hibernating material and deposited eggs in the field by that date. The parasite studies in the insectary, which were started with the deposition of the first eggs of the second brood on June 27, showed that the last parasite eggs that produced transforming parasites were deposited on September 15. The first eggs that produced wintering parasites were deposited on August 21. Thus, parasite eggs deposited between August 21 and September 15, inclusive, produced both transforming and wintering Macrocentrus. The last parasite eggs of the season, the progeny of which survived hibernation and emerged as adults the following spring, were deposited on November 2.

In 1928 it was possible to begin the studies in the insectary with both moths and parasites that emerged from hibernation in outdoor cages. The first Grapholitha eggs were deposited on April 25, and

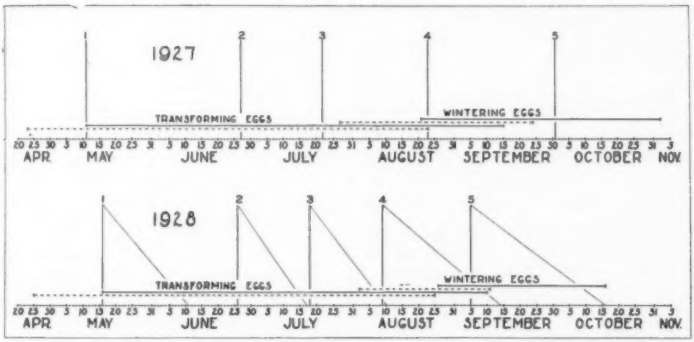

FIGURE 12.—Comparative periods during which eggs were deposited by *Macrocentrus ancylivorus* (solid line) and *Grapholitha molesta* (dotted line) and number of generations of *M. ancylivorus* in 1927 and 1928. The period of egg deposition for each generation of parasites in 1928 is indicated by the slanting line which reaches the base on the date the last egg was laid. The beginning of each generation is marked by a vertical line with the number of the generation at the top

the last eggs from which transforming fruit moths developed were deposited on August 25. Eggs producing wintering Grapholitha were deposited from August 2 to September 11, inclusive. Both transforming and wintering Grapholitha developed from eggs deposited from August 2 to August 25, inclusive.

Eggs that produced transforming parasites in 1928 were deposited from May 16 to September 10, inclusive, and eggs producing wintering parasites were deposited from August 26 to October 16, inclusive. Both transforming and wintering Macrocentrus developed from parasite eggs deposited between August 26 and September 10, inclusive.

NUMBER OF GENERATIONS

Five generations of *Macrocentrus ancylivorus* occurred during each of the seasons of 1927 and 1928. The dates on which the first eggs of each brood were deposited during each season are indicated in Figure 12. The period of egg deposition for each brood in 1928 is also shown, being indicated by the slanting line terminating at the

date on which the last egg was laid. Similar records were not available for 1927.

In 1927 the first and second broods were entirely transforming. All of the third-brood eggs, except a few of the latest ones deposited, produced transforming parasites. About two-thirds of the eggs of the fourth brood produced transforming individuals, and the remainder produced wintering ones. The fifth brood was entirely a wintering one. There were only four generations of the host species during that season.

In 1928 the first three broods of parasites were entirely transforming. Brood 4 contained a decided majority of transforming individuals, and brood 5, except for a few from the very earliest eggs that were deposited, produced wintering individuals. A period of about two weeks elapsed between the time of deposition of the last eggs of the first brood and the first eggs of the second brood in 1928. This was due to the fact that practically all the female parasites which emerged in the spring from hibernating material had died by the middle of June, and as an average life cycle of 41.3 days was required for development of the earliest individuals of the first brood, eggs of which were deposited on May 16, first-brood adults did not begin to emerge until about June 26. Thus, female parasites were not present in the insectary to deposit eggs from June 11 to June 26. A decided decrease in parasitism by *Macrocentrus ancylivorus* also occurred in the field during this same period in 1928, as indicated by weekly collections of Grapholitha larvae from infested peach twigs. These data have been presented in tabular form by the writer (*3, p. 373*). Parasitism by this species, which had reached a peak of 49.9 per cent in the field at the collection of May 29–June 1, gradually decreased to a minimum of 17.9 per cent for the period June 19–20. It then increased to 29.5 per cent for the period June 26–28, and the following week, July 3–5, it jumped to the high peak of the season, 67.9 per cent. Since the twigs were heavily infested by larvae of the host species throughout this entire period it is evident that the decrease in parasitism in the field was also due to a scarcity of female parasites during the two weeks immediately preceding the emergence of first-brood adults late in June. Five complete or partial generations of the host species occurred in 1928.

HIBERNATION

Macrocentrus ancylivorus hibernates as a first-instar larva within the body of the full-grown hibernating host larva. In the spring of 1929, a few weeks before wintering parasite larvae would begin to emerge from the host larvae, a number of hibernating larvae of the oriental fruit moth that had been parasitized by *M. ancylivorus* the previous fall were dissected. Each Grapholitha larva contained one live first-instar parasite larva and, in most instances, also several head capsules and bodies of dead parasite larvae. Measurements of the head capsules of the dead parasite larvae showed them to be of the same instar as the live ones.

During both 1927 and 1928 a considerable number of parasite larvae reached the cocoon stage late in the fall but failed to emerge as adults. Such cocoons were allowed to go through the winter undisturbed in order to ascertain whether any individuals might success-

fully hibernate within the parasite cocoon. In no instance did adults emerge in the spring from parasite cocoons spun the previous fall. The majority of these cocoons, when opened, were found to contain dead pupae, which suggests that these individuals pupated late in the fall but were unable to survive the sudden drops in temperature which occurred before they could emerge.

Considerable mortality after the parasite cocoon stage was reached also occurred in parasites which survived hibernation within the host and reached the cocoon stage in the spring. In 1928 and 1929 50 and 63 per cent, respectively, of the hibernating parasites that emerged from the host and spun cocoons in the spring failed to emerge as adults. Subsequent examination of these did not reveal the cause of death, although it was found that the majority had died as prepupae.

SUMMARY

Macrocentrus ancylivorus Roh. is an important parasite of the oriental fruit moth in the vicinity of Riverton, N. J. A closely related species, *M. delicatus* Cress., is parasitic on the same host species, but adults of *ancylivorus* and *delicatus* have distinguishing characters by which living specimens may be separated.

A greater percentage of female than of male parasites is produced when larvae of the oriental fruit moth and larvae of the strawberry leaf roller are parasitized by *Macrocentrus ancylivorus* in the field. A consistently greater proportion of females does not always occur when parasitism takes place under insectary conditions.

The average longevity of adult parasites of both sexes confined together in cages is 6.7 days; the average for unfertilized females confined alone is 17.9 days, and for males confined alone, 10.3 days.

Oviposition is possible only when the larva of the host species is in a position in which it can not escape the ovipositor of the female parasite. Superparasitism occurs, but only one parasite larva survives.

Unfertilized females produce only males.

Fruit-moth larvae of all sizes are subject to attack by this parasite. Parasitism in the field occurs chiefly in larvae of the early broods which feed in peach twigs.

As many as 40 offspring have been produced by a single female of *Macrocentrus ancylivorus* under insectary conditions.

The transforming parasite larva emerges from the host, on an average, 3 days after the Grapholitha larva would normally have pupated, and feeds on it for 12 to 18 hours. Wintering parasite larvae emerge from the host larvae in the spring and require an average of 2 days for feeding. About 24 hours are required for formation of the parasite cocoon. The combined prepupal and pupal period averages 15.2 days for transforming parasites and 38.5 days for wintering parasites. The average life cycle of transforming and wintering Macrocentrus is, respectively, 5.1 and 31 days shorter than the average life cycle of transforming and wintering Grapholitha.

Both adult parasites and moths emerge earlier in the spring from larvae which hibernate in outdoor cages than from larvae which hibernate in the insectary. In outdoor cages the spring emergence of the moths occurs considerably earlier than the spring emergence of the parasites.

During late summer and early fall there is a period during which eggs deposited by Macrocentrus and by Grapholitha may produce both transforming and wintering progeny.

There were five generations of this parasite in the Riverton-Moorestown area in 1927 and in 1928.

Macrocentrus hibernates as a first-stage larva within the full-grown hibernating larva of the oriental fruit moth. Many parasite cocoons that are constructed late in the fall and in the spring fail to produce adult parasites owing to mortality of the prepupae and pupae.

LITERATURE CITED

(1) Driggers, B. F., and Pepper, B. B.
1931. Macrocentrus ancylivora Rohr. and M. delicatus Cress. distinct species. Ann. Ent. Soc. Amer. 24:293–301, illus.

(2) Fink, D. E.
1926. The biology of Macrocentrus ancylivora Rohwer, an important parasite of the strawberry leaf roller (Ancylis comptana Froehl.). Jour. Agr. Research 32:1121–1134, illus.

(3) Haeussler, G. J.
1930. Parasites of the oriental peach moth, Laspeyresia molesta Busck, in North America. Jour. Agr. Research 41:365–377, illus.

(4) Peterson, A., and Haeussler, G. J.
1926. The oriental peach moth. U. S. Dept. Agr. Circ. 395, 28 p., illus.

(5) ———and Haeussler, G. J.
1928. Determination of the spring-brood emergence of oriental peach moths and codling moths by various methods. Jour. Agr. Research 37:399–417, illus.

(6) ——— and Haeussler, G. J.
1930. Life history of the oriental peach moth at Riverton, N. J., in relation to temperature. U. S. Dept. Agr. Tech. Bul. 183, 38 p., illus.

(7) Rohwer, S. A.
1923. A new Macrocentrus reared from the strawberry leaf-roller (Hymenoptera, Braconidae). Ent. Soc. Wash. Proc. 25:168.

(8) Stearns, L. A.
1928. The larval parasites of the oriental peach moth (Laspeyresia molesta Busck) with special reference to the biology of Macrocentrus ancylivora Rohwer. N. J. Agr. Expt. Sta. Bul. 460, 24 p., illus.

EFFECT OF SIZE OF CROWN AND LENGTH OF CUTTING SEASON ON YIELDS OF ASPARAGUS [1]

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INTRODUCTION

Plants of *Asparagus officianalis* L. produced from field-run seed vary potentially from very poor yielding to very good yielding, the largest number being intermediate in yield. Nurserymen selling asparagus crowns usually grow them from seed planted in a nursery row. The seedlings are allowed to remain in the row for one or two years before they are transplanted to the permanent plantation. Many 1-year-old crowns are small, especially if the seed is sown too thick. These small roots may have genetic potentialities for larger yields. Should they be discarded or will they produce satisfactory yields?

Root selection in asparagus has been advocated for a number of years. In selecting for size, however, there is always the possibility that crowns discarded because of their smallness might have produced satisfactory yields had they been planted in the permanent location. The small size might have been due entirely to environmental conditions in the nursery row, such as crowding, poor soil, or lack of moisture, and not to factors of heredity. Under favorable conditions for growth the plant might have reached normal size and, since cutting is usually delayed for two years after the crowns are planted, the small crowns might have yielded as well as the large ones.

The purpose of the investigation herein reported was to determine (1) the effect of size of root at planting on the subsequent yield of the plant, and (2) the effect of length of cutting season on yields.

REVIEW OF LITERATURE

Robbins and Jones (*6*, *7*) [2] found that staminate plants outyield pistillate plants. This difference in yield is greater early in the season than later, and the pistillate spears are, on an average, larger than the staminate spears. This confirms the findings of Green (*2*) and Tiedjens (*9*).

Myers (*5*) reports the yields of three grades of crowns for a period of six years. He found little difference in yield between first and second grade crowns, but small crowns produced yields decidedly inferior to the first two. Jones and Robbins (*4*) found that desiccated crowns produced 12.4 per cent less the first year and 27 per cent less the second year than nondesiccated crowns. Unpruned crowns outyielded pruned crowns by 14 per cent the second season. The same authors (*3*) concluded that no injury resulted from harvesting spears for a short time the year after planting. Schermerhorn (*8*) concluded that the diameter of brush did not necessarily indicate the diameter

[1] Received for publication Dec. 14, 1931; issued August, 1932. Journal Paper No. B 22 of the Iowa Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 109.

of the spears that might be cut the following spring. A more accurate index of plant performance is the actual diameter and number of spears per plant.

RELATION OF SIZE OF CROWN TO YIELD

In 1927 an experiment was started to determine what effect the size of the roots at planting time would have on the yield of the plants. One hundred and fifty 1-year-old crowns were dug from the nursery row in the spring before growth started. These were taken to the laboratory immediately, washed free of earth, surface-dried with a soft cloth, weighed, and immediately planted in the field.

The roots were not selected but were taken at random from the nursery row. They ranged in weight from 3 to 57 grams. They were planted 4 by 2 feet in 1927, and no cuttings were made until the spring of 1929. When the plants bloomed, it was found that there were 79 male and 71 female crowns. The records of cuttings were kept for each plant, the total weight of spears and the number of spears produced at each cutting being recorded. The spears were cut when 6 to 8 inches high. Cuttings were made until June 15 each year.

Unless individual yield records have been kept for asparagus plants, it may be thought that an asparagus plant yields continuously at every cutting throughout the season. This is not the case with every plant, and very few plants yield marketable spears at every cutting. Table 1 gives the individual yield records of some high and some low yielding plants for 1931. It may be seen from this table that many plants yield very little throughout the season, while others are comparatively heavy yielders. The roots of the heavy-yielding plants averaged 20 grams each when planted and those of the low-yielding plants averaged 16 grams each.

In Table 2 are presented the number of spears, total weight of spears, and average weight of spears for each sex for the first three cutting seasons.

Male crowns produce a greater number of spears and a heavier yield per plant than female crowns. On the other hand, female crowns produce spears of larger diameter, as is indicated by the average weight of spears. The average weight of the male crowns at planting was slightly greater than that of the female crowns. However, if all crowns that weighed 15 g or less had been discarded, more male crowns would have been discarded than female crowns. These observations are only incidental to the main problem and are in harmony with the results reported by other investigators.

The question arises as to the advisability of planting all male plants rather than both male and female. According to Robbins and Jones (*6*), asparagus seedlings in California frequently bloom in September of the year in which the seed is sown, so that it is possible to select 1-year-old male plants, but in the Northern States plants do not bloom the first year and a few do not bloom the second year. One-year-old crowns are superior to 2-year-old crowns, so that it would not pay, probably, to grow them two years to determine the sex.

TABLE 1.—*Yields of certain low and high producing asparagus plants, on various dates in 1931*

[The figure before the dash represents the number of spears, and the figure after the dash, the weight of the spears in grams]

Plant No.	Apr. 18	Apr. 24	May 2	May 5	May 15	May 19	May 23	May 25	May 27
15	3—60	1—8	2—58	1—24	3—56	3—62	3—62	1—40	
28		3—20	8—118	4—96	5—120	3—52	2—18	5—96	3—28
46		8—90	2—16	5—42	5—40	6—80	3—90	1—10	7—62
102		3—126	2—64	4—112	4—232	1—36	2—60	2—54	
106	6—46	1—8	9—84	3—44	10—296		3—82	1—18	4—32
118	1—14		8—102	4—80	2—42	6—180	2—20	3—52	2—26
31					1—16				
44		1—20	1—8	1—10		1—12			
61							1—30		
119				1—24		1—24			
120						1—26			
115				1—32	1—18	2—66			1—26
89				1—68	1—52	1—22			2—112
92						1—18			

Plant No.	May 29	June 1	June 4	June 6	June 9	June 11	June 13	Total number of spears	Total weight of spears
15		1—8	2—36	2—48	4—54	1—18		27	534
28	2—26		9—258	1—10	5—72	2—16	4—64	56	994
46		3—32	1—8	6—112		4—42	6—56	57	680
102	4—106		2—32	1—24		3—62	2—52	30	960
106	4—66	2—62	1—16	6—94		5—48		55	896
118	1—16	3—48	5—130	3—58	3—38	1—16	2—16	46	838
31			1—18			1—24		3	58
44				1—8		1—12		6	70
61		1—26				1—6	1—20	4	82
119				1—18				3	66
120		1—26		1—20				3	72
115		1—28		2—106				8	276
89		1—18				1—76		7	348
92		1—14						2	32

TABLE 2.—*Yields of male and female asparagus plants, 1929–1931*

[The standard deviation rather than the probable error follows each mean]

Plants	Average weight of crowns when planted	1929		
		Average weight of spears	Spears per plant	Total weight per plant
	Grams	*Grams*	*Number*	*Grams*
Male	22.11±1.74	17.35±0.57	9.59±0.52	162.83±8.59
Female	20.46±1.87	17.68± .43	7.02± .35	119.78±5.90
Average	21.68±1.78	17.49± .56	8.48± .47	144.25±7.36

Plants	1930			1931		
	Average weight of spears	Spears per plant	Total weight per plant	Average weight of spears	Spears per plant	Total weight per plant
	Grams	*Number*	*Grams*	*Grams*	*Number*	*Grams*
Male	16.82±0.55	15.37±0.88	246.68±12.28	18.74±0.15	21.33±0.97	393.35±3.92
Female	21.09± .51	9.39± .05	184.44± 8.91	21.56± .05	11.05± .58	248.17±2.95
Average	18.66± .53	12.79± .73	219.82±10.96	19.96± .09	16.89± .78	332.37±3.53

On the top line, or line A, of Table 3, are presented the correlation coefficients between weight of crown at planting and average weight of spears; weight of crown and number of spears per plant; and weight of crown and total weight per plant for the years 1929, 1930, and 1931. The correlation coefficients were calculated separately for the male and female plants and the total correlation coefficients presented in Table 3 were secured by pooling according to Fisher's (*1*) method, which gives the same benefit as the use of a larger number of observations.

In lines B, C, D, etc., Table 3, are presented the correlation coefficients between average weight, total weight, and number of spears for three years. These were obtained as a matter of interest and have no bearing on the coefficients between crown weight and other variables presented in line A. The tests of significance were taken from Wallace and Snedecor's (*10*) table of significant values of r, R, and t adapted from Fisher's (*1*), tables of t and z.

TABLE 3.—*Correlation coefficients for asparagus Plants, 1929–1931*

MALE PLANTS

A	B	C	D	E	F	G	H	I	J
Weight of crown	Average weight of spears, 1931	Number spears per plant, 1931	Total weight per plant, 1931	Average weight of spears, 1930	Number spears per plant, 1930	Total weight per plant, 1930	Average weight of spears, 1929	Number spears per plant, 1929	Total weight per plant, 1929
A	0.0135	[a]0.2745	[a]0.2521	0.0439	[a]0.2699	[a]0.3101	0.0659	[b]0.3691	[b]0.3447
B		−.0816	[b].6148	−.1195	−.0639	.2414	[b].4135	.1587	.2469
C			[b].8874	.0889	[b].9016	[b].7779	−.0146	[b].7113	[b].6263
D					[b].7945	[b].8472	.1317	[b].7657	[b].7556
E					−.1843	[a].3043	[b].6162	−.0329	[a].2641
F						[b].8259	−.0882	[b].7029	[b].5464
G							.2158	[b].7166	[b].7430
H								−.0270	[b].3700
I									[b].8736

FEMALE PLANTS

A	0.0167	−0.1808	−0.1725	−0.0275	[a]−0.3024	[a]−0.3281	0.0201	−0.1848	−0.1771
B		−.0566	[a].3648	[b].5388	−.0550	[a].3279	[b].5858	−.1167	.1503
C			[b].7255	−.1945	[b].7475	[b].6639	−.1226	[b].7785	[b].6008
D				.1001	[b].6106	[b].7582	.0540	[b].6007	[b].5279
E					[a]−.3134	.2378	[b].6031	−.1342	.1991
F						[b].8047	−.2002	[b].7567	[b].5518
G							.1623	[b].6510	[b].6465
H								.1294	[b].4387
I									[b].8075

ALL PLANTS

A	0.0147	0.1603	0.1139	0.0082	0.1241	0.0074	0.0465	0.1818	0.1577
B		−.0622	[b].2919	[b].5678	−.0531	[b].2717	[b].4986	.0331	[a].2301
C			[b].8453	−.1233	[b].8787	[b].7259	−.0388	[b].7092	[b].6024
D				.0878	[b].7521	[b].8172	.1025	[b].7217	[b].6889
E					[a]−.2039	[b].2640	[b].6026	−.0726	[a].2250
F						[b].8017	−.0114	[b].7069	[b].5378
G							.1934	[b].6944	[b].7107
H								.0629	[b].3927
I									[b].8530

[a] Significant. [b] Highly significant.

The average weight of spears was not correlated with size of crown in male or female plants in any of the three years' data recorded. Total yield or total weight of spears was significantly correlated with weight of crown in male plants for three years; in 1929 the correlation

coefficient was highly significant. With the female plants there was a negative correlation between yield and weight of crown, but in 1930 only was this negative correlation significant, and then only slightly so. The correlation coefficients between number of spears per plant and weight of crown were slightly significant in male plants, but only one significant negative correlation was found in female plants.

There are no significant correlations between average weight of spears and number of spears in either sex except in the female plants in 1930, when a slightly significant correlation was found. Total weight of spears per plant and number of spears are highly correlated in the same year, or what one might expect the following year or two years later.

Total weight of spears in any one year is highly correlated with total weight of spears in each of the other two years, and the same relationship holds true for number of spears and average weight of spears for either sex; i. e., if one secures a high yield or a large number of spears, or spears of large diameter from crowns for one season, one may expect approximately the same relationship in following years.

EFFECT OF THE LENGTH OF THE CUTTING SEASON ON YIELDS

A matter of interest to professional as well as to amateur gardeners is the season of year and the length of time asparagus may be cut without detriment to future yields. Since there is a ready sale for the product quite late in the summer, this matter is important.

To determine the effect of length of cutting season on yields, a series of plots was planted in 1927. The plants were set 2 feet apart in rows 4 feet apart and 100 feet long. The soil was Webster silt loam, and no fertilizers were applied either before or after planting. The first cuttings were made in 1929. Three years' records, 1929, 1930, and 1931, are now available. Each year rows were cut as follows: 6 rows were cut up to and including May 1; 8 rows were cut up to and including May 15; 8 rows were cut up to and including June 1; 8 rows were cut up to and including June 15; 6 rows were cut up to and including July 1; and 6 rows were cut up to and including July 15. The rows to be cut were selected at random at the beginning of the experiment. They were not located in a solid block but were scattered through the field.

In Table 4 are presented the average total yield and the average number of spears for the replicates in each series.

TABLE 4.—*Average yield and average number of spears of the asparagus replicates for each date of cutting*

Date when cutting ceased		Weight of asparagus cut	Spears cut	Date when cutting ceased		Weight of asparagus cut	Spears cut
		Ounces	*Number*			*Ounces*	*Number*
May 1	1929	34.3± 2.44	79.0± 5.58	June 15	1929	290.0±14.75	496.9±24.42
	1930	46.8± 2.88	55.5± 4.10		1930	262.5±13.39	479.7±24.71
	1931	93.0±13.46	150.8±20.10		1931	470.8±25.12	795.3±25.12
May 15	1929	83.4± 4.32	159.0± 7.50	July 1	1929	388.8±19.37	672.0±30.50
	1930	174.9±10.32	238.1±11.24		1930	360.8±10.22	678.3±26.81
	1931	191.8± 7.64	243.7±11.60		1931	554.3±19.89	975.2±44.03
June 1	1929	209.0± 4.58	349.7± 7.19	July 15	1929	413.2±32.60	770.5±42.43
	1930	217.0± 5.18	343.5± 9.93		1930	393.5±17.75	678.5±29.71
	1931	342.5± 5.97	533.0±11.82		1931	408.2±41.45	925.0±64.96

All rows cut in 1931 showed an increase in yield over the preceding year, although this increase was slight for rows cut up to and including July 15. All rows showed an increase in number of spears irrespective of length of cutting season in 1931. A slight decrease in yield irrespective of treatment in 1930 as compared to 1929 occurred in rows cut up to and including June 15, July 1, and July 15. Rows cut until May 1, May 15, and June 15 showed only a very small increase in 1930 as compared to 1929. This small increase may be accounted for by the fact that three very heavy freezes occurred after the beginning of the growing season, and it was impossible to obtain yield records for this period since all spears were killed. Also, the severe drought of 1930 affected the June 15, July 1, and July 15 plots. Rows cut until July 15 in 1931 showed a decidedly smaller yield than rows cut until June 15 or July 1, even though the cutting season extended two weeks and one month longer, which represented 7 and 13 more cuttings, respectively.

In Table 5 the mean differences between yields and number of spears are presented along with the probability (P) values secured from Fisher's (t) table of t. There is a significant difference in yields between rows cut until July 1, 1931, and those cut until June 15, 1931. In other words, the yield was greater by cutting longer, and apparently cutting until July 1 in 1929 and 1930 did not cause a decrease in yield in 1931. On the other hand, rows cut until July 15, 1931, did not yield as much as those cut until June 15, although 13 more cuttings were made. The long cutting season in 1929 and 1930 (to July 15) seriously affected the yielding capacity of the rows in 1931, since the value of P is not significant between the mean values of June 15 and July 15.

TABLE 5.—*Mean differences (M. D.) between yields and number of spears, with probability values (P) for asparagus plots cut at different dates in 1931*

Item	M. D.	P	Significance
DIFFERENCE BETWEEN PLOTS CUT UNTIL JUNE 15 AND JULY 1			
Yield.....ounces..	83. 5±33. 87	0. 05−0. 02	Significant.
Spears.....number..	179. 9±62. 84	.02− .01	Do.
DIFFERENCE BETWEEN PLOTS CUT UNTIL JUNE 15 AND JULY 15			
Yield.....ounces..	−62. 6±45. 96	0. 2−0. 1	Not significant.
Spears.....number..	129. 7±74. 91	.2− .1	Do.
DIFFERENCE BETWEEN PLOTS CUT UNTIL JUNE 1 AND JUNE 15			
Yield.....ounces..	128. 3±25. 83	(*)	Highly significant.
Spears.....number..	262. 3±44. 73	(*)	Do.

* Less than 0.1.

TABLE 6.—*Analysis of variance*

TREATMENT OF YIELDS

Variance	Degrees of freedom [a]	Sum of squares	Mean squares	Standard deviation	Log S. D.[a]
Between classes	3	101,370.5816	33,790.1739	183.821	5.2185
Within classes	24	37,383.256	1,557.6356	39.4669	3.6815
Total	27	138,753.8376	5,139.031	71.6870	[b] 1.5370

TREATMENT OF NUMBER OF SPEARS

Between classes	3	133,894.053	44,631.351	211.2613	5.2598
Within classes	24	103,563.439	4,315.143	65.6897	4.1914
Total	27	237,457.492	8,794.721	93.780	[b] 1.1684

[a] 5 per cent point of the distribution of $Z=0.5508$; 1 per cent point=0.7757. [b] Difference.

Do rows cut until June 1 yield as well as those cut until June 15? Perhaps cutting even till the middle of June may have caused some decrease in yield in 1931. However, since the value of P is highly significant, it is evident that the yield was increased by cutting until June 15.

It was thought well to determine what the rows yielded in 1931 when yield records were taken to a definite date; in other words, the yields were determined to June 1 for rows that had been cut a longer period during the three years. The same statistical treatment was used for yields to June 15 for rows cut longer than this period. To determine whether differences in weight and number of spears were due to differences in length of cutting season or whether they were due to random sampling, Fisher's (*1*) analysis of variance was used in a statistical treatment of the data. (Table 6.)

The values for the 5 per cent and 1 per cent points of the distribution of z (taken from Fisher's (*1*) table of z) for the number of degrees of freedom involved when compared with the difference of the logarithms of the standard deviations, indicate that the differences obtained probably are due to the treatment rather than to random sampling.

In Table 7 the weight and number of spears to June 15 for rows cut after this date are treated by analysis of variance. The mean differences between June 15 and July 1 rows (cut until June 15, 1931) are not significantly different since the values of P are not significant. Mean differences between June 15 and July 15 rows (cut until June 15, 1931) are significantly different, as are the mean differences between July 1 and July 15 rows (cut until June 15, 1931). From this it may readily be seen that cutting until July 15 is entirely too long for maximum yields in the following years.

In Table 8 the weight and number of spears to June 1 for rows cut after this date are treated as in Table 7. No significant differences were found to June 1, 1931, between rows cut until June 1 and June 15 during the two preceding years, nor were there significant differences between June 1 and July 1 rows in yield and number of spears to June 1, 1931. On the other hand, highly significant differences were obtained between June 1 and July 15 rows cut to June 1, 1931, and also between July 1 and July 15 rows cut to the same date. By taking the yeilds to a definite date for all rows, a comparison may be made which includes the same number of cuttings for all treatments.

TABLE 7.—*Mean differences (M. D.) between yields and number of spears, with probability values (P) for asparagus plots cut at different dates in 1931*

Item	M. D.	P	Significance
DIFFERENCE BETWEEN JULY 1 AND JUNE 15 PLOTS CUT UNTIL JUNE 15			
Yield.....ounces..	55.3±30.91	0.2—0.1	Not significant.
Spears.....number..	90.0±57.24	.2— .1	Do.
DIFFERENCE BETWEEN JULY 15 AND JUNE 15 CUT UNTIL JUNE 15			
Yield.....ounces..	201.5±37.06	[a]	Significant.
Spears.....number..	249.5±59.44	[a]	Do.
DIFFERENCE BETWEEN JULY 15 AND JULY 1 CUT UNTIL JUNE 15			
Yield.....ounces..	146.2±28.93	[a]	Significant.
Spears.....number..	159.5±48.99	[a]	Do.

[a] Less than 0.01.

TABLE 8.—*Mean differences (M. D.) between yields and number of spears, with probability values (P) for asparagus plots cut at different dates in 1931*

Item	M. D.	P	Significance
DIFFERENCE BETWEEN JUNE 1 AND JUNE 15 PLOTS CUT UNTIL JUNE 1			
Yield.....ounces..	15.625±20.0066	0.5—0.4	Not significant.
Spears.....number..	47.875±32.4579	.2— .1	Do.
DIFFERENCE BETWEEN JUNE 1 AND JULY 1 PLOTS CUT UNTIL JUNE 1			
Yield.....ounces..	36.667±23.4149	0.2—0.1	Not significant.
Spears.....number..	32.000±42.0680	.5— .4	Do.
DIFFERENCE BETWEEN JUNE 1 AND JULY 15 PLOTS CUT UNTIL JUNE 1			
Yield.....ounces..	143.833±28.4288	[a]	Highly significant.
Spears.....number..	148.333±42.4404	[a]	Do.
DIFFERENCE BETWEEN JULY 1 AND JULY 15 PLOTS CUT UNTIL JUNE 1			
Yield.....ounces..	107.165±19.0848	[a]	Highly significant.
Spears.....number..	116.333±36.0741	[a]	Do.

[a] Less than 0.01.

SUMMARY AND CONCLUSIONS

Since it is usually impossible to determine the sex of 1-year-old asparagus plants in the Northern States, and since the weight of female crowns average slightly less than that of male crowns, it might be well to discard very small crowns because of the possibility that by so doing a large number of male crowns would be retained.

The results of the correlation studies herein reported confirm the conclusions of several other workers in showing that male crowns produce a greater number of spears and a heavier yield per plant than female crowns and that female crowns produce spears of larger diameter.

Under the conditions of these experiments significant correlations exist in male asparagus plants between the weight of 1-year-old crowns when planted and the number of spears and total weight of

spears produced during the first three cutting seasons. There were no significant correlations between the weight of the crown at planting and the average weight of the spears produced. Female plants during 1930 showed a significant negative correlation between weight of crown and total number of spears and total yield; however, in the preceding year and in the following year no such correlation existed. When male and female plants are pooled, no significant correlation coefficients are found between these factors.

For the three years' data recorded there seems to be an increase in weight and number of spears for all rows irrespective of length of cutting season, except rows cut until July 15. There is an increase in production for 1931 in each successive series except for rows cut until July 15. Rows cut until July 15 in the third year did not yield as much as rows cut until June 15, although 13 more cuttings were made. Rows cut until July 15 did not yield as much as the rows cut until July 1, although 7 more cuttings were made. Cutting until July 1 and June 15 in 1931 greatly increased the crop produced as compared with rows cut until June 1, 1931.

June 1, June 15, and July 1 rows yielded to June 1, 1931, approximately the same amount; at least no significant differences were noted; as compared with these same series, the July 15 rows cut to June 1, 1931, showed a significant decrease in yield. Under the conditions of this experiment, three years' cutting records demonstrate that July 15 is entirely too long to harvest asparagus. The rows cut until July 1 showed no decrease in yield, but the increases over the rows cut a shorter period of time though significant are not highly so, and future records may disclose that this date is entirely too late to harvest.

LITERATURE CITED

(1) Fisher, R. A.
1930. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 p., illus. Edinburgh and London.
(2) Green, W. J.
1891. ASPARAGUS. Ohio Agr. Expt. Sta. Bul. ser. 2, v. 3, p. [241]–244.
(3) Jones, H. A., and Robbins, W. W.
[1927]. INFLUENCE OF CUTTING ASPARAGUS THE FIRST YEAR AFTER PLANTING ON PRODUCTION THE FOLLOWING YEAR. Amer. Soc. Hort. Sci. Proc. (1926) 23: 23–25.
(4) ——— and Robbins, W. W.
[1927]. INFLUENCE OF DESICCATION AND ROOT PRUNING ON PERFORMANCE OF ASPARAGUS. Amer. Soc. Hort. Sci. Proc. (1926) 23: 26–28.
(5) Myers, C. E.
1918. EXPERIMENTS WITH ASPARAGUS. Penn. Agr. Expt. Sta. Ann. Rpt. 1915–16: 557–578.
(6) Robbins, W. W., and Jones, H. A.
1925. SECONDARY SEX CHARACTERS IN ASPARAGUS OFFICIANALIS L. Hilgardia 1: [183]–202, illus.
(7) ——— and Jones, H. A.
1929. FURTHER STUDIES OF SEX IN ASPARAGUS. Amer. Soc. Hort. Sci. Proc. (1928) 25: 13–16, illus.
(8) Schermerhorn, L. G.
1929. A SUMMARY OF THE PERFORMANCE RECORDS OF INDIVIDUAL ASPARAGUS PLANTS IN 1928. Amer. Soc. Hort. Sci. Proc. (1928) 25: 35–36.
(9) Tiedjens, V. A.
[1925]. SOME PHYSIOLOGICAL ASPECTS OF ASPARAGUS OFFICIANALIS. Amer. Soc. Hort. Sci. Proc. (1924) 21: 129–140.
(10) Wallace, H. A., and Snedecor, G. W.
1931. CORRELATION AND MACHINE CALCULATION. Rev. by G. W. Snedecor. Iowa Agr. Col. Off. Pub. v. 34, no. 4, 71 p.

THE VALUE OF IODINE FOR LIVESTOCK IN CENTRAL PENNSYLVANIA[1][2]

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INTRODUCTION

A series of feeding experiments was conducted at the Pennsylvania State College in cooperation between the college and the Mellon Institute of Industrial Research—the latter acting through the multiple industrial fellowship of the Iodine Educational Bureau—for the purpose of studying the value of iodine when fed to farm animals, in a manner supplementary to ordinary rations, under conditions present in central Pennsylvania.

In relation to the iodine content of feeding stuffs and waters, central Pennsylvania is sometimes spoken of as a border-line district, in the sense that the needs of domestic animals for iodine are ordinarily satisfied, while an occasional appearance of goiter or of hairlessness in young animals shows that the usual margin of safety in the supply of iodine is so small that a deficiency sometimes occurs.

The essential relation of iodine to the function of the thyroid gland being thoroughly established, and deficiency of iodine in food or water having been shown to be at least a major cause of disturbance of thyroid function, a special interest attaches to the iodine problem in regions such as central Pennsylvania in which there is no acute, general iodine deficiency, since it is not known that supplementary iodine is of value only in regions in which the iodine content of foodstuffs and waters is so deficient as to manifest itself through the prevalence of goiter and related disorders.

In other words it is at least logically possible that iodine might have a nutritive value in relations other than with the thyroid, and the special object of this study was to throw light on this possibility.

SCOPE OF INVESTIGATION

The scope of the investigation was as follows: (1) A feeding experiment was conducted with 25 Holstein-Friesian cows which were infected with *Brucella abortus*, and which were individually subjected to maximum dosage with iodine in an effort to learn the effects of such treatment on the course of the abortion disease; (2) a feeding experi-

[1] Received for publication Dec. 16, 1931; issued August, 1932.

[2] The general outline of this project was proposed by E. B. Forbes, who also maintained direct oversight of the experimental work and the preparation of the results for publication. Geo. M. Karns devised and prepared the iodine supplement employed, performed the iodine estimations on feedstuffs and drinking water, and cooperated in directing the experiment, through correspondence and personal visits. S. I. Bechdel and P. S. Williams conducted (1) a feeding experiment on cows infected with *Brucella abortus*, to determine the effect of the maximum dosage of iodine on the course of the disease, and (2) a feeding experiment with calves to determine the value of supplementary iodine for growth. T. B. Keith conducted experiments with swine, and also with lambs fed as matched pairs or in groups, to learn the value of supplementary iodine in their growth. E. W. Callenbach and R. R. Murphy conducted a feeding experiment with chickens to learn the effects of iodine on growth and early egg production.

ment with 42 Holstein-Friesian calves, fed as 21 matched pairs, to determine the value of supplementary iodine for growth; (3) a feeding experiment with 40 swine, fed as 10 lots of 4 individuals each; (4) a feeding experiment with 30 lambs fed as 15 matched pairs, and a feeding experiment with 171 lambs, fed as 6 lots of 28 or 29 individuals each, to learn the value of supplementary iodine in the growth of these animals; and (5) a feeding experiment with 701 chickens, fed in 4 groups of 160 to 187 each, to learn the effects of iodine in the growth and early egg production of this species.

In all, therefore, 1,009 animals were fed in this investigation.

The iodine was administered in nearly the whole of this work in the form of a new preparation—iodized linseed meal. This was elemental iodine so combined with linseed meal as to leave but a trace of free iodine.

In the preparation of this compound a certain quantity of old-process linseed meal is placed in a tumbling mill with a known quantity of iodine. The two are then intimately mixed for about 12 hours, by the end of which time the meal and the iodine have become uniformly mixed and a reaction between the two has begun. The preparation is then stored under such conditions that uncombined iodine will not be lost, and the reaction between the two components is completed within a few days. The meal is then standardized as to iodine content.

This preparation was made several times during the course of the experiments, the several lots being standardized to contain from 4 to about 10 per cent of iodine, and the iodine was fed in this form in all the experiments except during a very small portion of the work with calves.

The results of these experiments therefore apply with certainty only to the particular preparation of iodine which was employed, and the reaction of the animals to dosage with this preparation rendered perfectly clear the fact that their tolerance for iodine in this preparation is much less than for iodine as potassium iodide.

The dosage of iodine employed, expressed in milligrams of iodine per day, per 100 pounds live weight, was as follows: Calves, 20; sheep, 30; swine, 50; chickens, 50.

This dosage, which may properly be designated as therapeutic rather than nutritional, in view of the minuteness of the quantities of iodine involved in the functioning of the thyroid gland, was arrived at after a review of published reports on other feeding experiments in which iodine was administered in quantities greater than those required for the maintenance of thyroid function. It is not known that the liberality of the dosage was in any way prejudicial to the state of nutrition of the experimental subjects.

These experiments were conducted with a certain degree of consideration of the locality involved, since the geographic distribution of iodine is significant in relation to animal nutrition; but it was considered more desirable to feed the animals in a manner representative of prevailing custom in the section than to restrict them to locally grown feeds, since central Pennsylvania does not depend in the main on feeding stuffs produced within this section, and it was not practicable to feed these animals as they are normally fed and at the same time feed them exclusively on locally produced products.

The rations, therefore, were composed not only of local products but also, to a large extent, of feedstuffs purchased in the market and of unknown geographic origin. The experiments were all conducted in dry lots. Had they been conducted on pasture the local influence would have been much more pronounced.

The iodine content of the feeding stuffs employed is given in Table 1. In general the iodine content of this group of products does not vary greatly, but as exceptions to this statement the oyster shell was appreciably richer in iodine than were the feeds of agricultural origin; the poultry mashes were also rich in iodine, probably on account of the iodine content of the cod-liver oil contained; and the grain mixture fed to calves contained very much more iodine than is known to be characteristic of any of the feedstuffs of which they are composed. Unfortunately it was impracticable to determine whether some one of the constituent feeding stuffs was responsible for this surprisingly high iodine value, or whether it was due to some unaccountable contamination. In all cases the basal ration contained iodine in quantities sufficient for the maintenance of thyroid function, as estimated from observations by Marine and Kimball on dogs.[3]

TABLE 1.—*Iodine content and origin of feeding stuffs and water used in various feeding experiments with different kinds of farm animals*

Subjects of experiments	Feeding stuff	Origin	Iodine, parts per billion[a]
40 swine	Linseed meal	Market, unknown origin	90.3
	Digester tankage	do	118
	Corn (maize), grain	do	55.5
	Oats, grain	do	104
	Wheat middlings	Local mill	
	Salt	Scranton, Pa.	130
	Water	Spring Creek	1.8
42 calves	Grain mixture	Part local, part market	27,300
	Timothy hay	Kylertown, Pa.	115
	Alfalfa hay	New Jersey	78
	Mixed hay	Kylertown, Pa.	60
	Clover hay	Ohio	61.8
	Skim milk	Local	70.7
	Salt	Scranton, Pa.	130
	Water	College well	1.1
171 lambs	Corn (maize), grain	Market, unknown origin	55.5
	Linseed meal	do	90.3
	Mixed hay	Local	150
	Rock salt	Scranton, Pa.	165
	Water	College well	1.1
30 lambs	Corn	Market, unknown origin	55.5
	Linseed meal	do	90.3
	Alfalfa hay	Local	78
	Salt	Scranton, Pa.	130
	Water	Spring Creek	1.8
701 chickens	Mash Nos. 1 and 2	Market, Buffalo, N. Y.	1,040
	Mash Nos. 3 and 4	do	1,090
	Scratch grains	do	19.4
	Semisolid buttermilk	Local	46
	Oyster shell	Local market	444
	Grit	do	44.5
	Water	College well	1.1

[a] Dry basis, except for waters.

[3] MARINE, D., and KIMBALL, O. P. THE PREVENTION OF SIMPLE GOITER IN MAN. A SURVEY OF THE INCIDENCE AND TYPES OF THYROID ENLARGEMENTS IN THE SCHOOLGIRLS OF AKRON (OHIO) FROM THE 5TH TO THE 12TH GRADES, INCLUSIVE—THE PLAN OF PREVENTION PROPOSED. Jour. Lab. and Clin. Med. 3: [40]–48, illus. 1917.

OBSERVATIONS ON THE EFFECTS OF ADMINISTERING IODINE TO COWS INFECTED WITH BRUCELLA ABORTUS

In the study to determine the reaction of cows affected with abortion disease to heavy dosages of iodine, the 25 cows used as subjects were from a herd of grade Holstein-Friesian cattle, maintained for experimental purposes on college farm No. 12, which is 2½ miles from the main dairy barn of the college.

Regular periodic blood tests revealed that this herd was free from abortion disease until the summer of 1929. At this time an outbreak of infectious abortion occurred, and vigorous steps were at once taken to prevent its spread. All reacting or suspected animals were removed to a quarantine barn about 2 miles away, but in spite of the fact that blood tests were made every two weeks, it seemed impossible to prevent a rapid spread of the disease throughout the herd. In fact 31 of the 40 cows reacted to the blood test before the spread of the disease was finally stopped.

As the plan of management included the holding of the reacting animals in quarantine for some time, an excellent opportunity was afforded for the experimental administration of iodine to infected cows and to some others which had been exposed but which had not reacted to the agglutination blood test.[4]

Iodized linseed meal was given, therefore, with the regular ration, in as large quantities as the animals would eat without going off feed. The heroic dosage employed was based on recommendations for the treatment of actinomycosis by administration of potassium iodide.

The rations of 11 of the cows (Nos. 5, 8, 12, 13, 16, 17, 18, 21, 32, 38, and 41) consisted of a grain mixture containing 17.5 per cent of protein, corn silage and alfalfa hay; while the ration of the remaining 14 cows consisted of a grain mixture containing 24 per cent of protein, corn silage and timothy hay.

Each of the cows was 3 years of age, and the greater number of them were in their second pregnancy.

NOTES ON INDIVIDUAL COWS RECEIVING IODINE

Cow No. 5 between December 4 and December 12 received 17 g of iodine; agglutination tests previous to July 17 were all negative; reaction was positive July 17, August 23, October 14, November 14, November 19, December 4, and December 13. The cow carried her calf until she was slaughtered December 13; she was due to freshen February 19. This cow received iodine eight days only.

Cow No. 8 between December 4 and January 1 received 51 g of iodine; agglutination tests were negative until April 1, when the test was doubtful; the reaction was positive April 15, negative May 1, doubtful July 17 and August 23, negative October 14 and 31, and November 14, and highly suspicious December 3. The cow calved on the date due, December 22; the calf was normal; the reaction was suspicious December 17, and slightly suspicious December 31. This cow reacted positively only once, on April 15, over seven months before iodine feeding was begun. Samples of the placenta and amniotic fluid were subjected to a test for Bang bacillus by injection into guinea pigs. The test showed that the organism was not present. This cow was heavily dosed with iodine for the 18 days just previous to a normal parturition.

Cow No. 10 between October 25 and November 12 received 16.47 g of iodine; agglutination tests previous to July 17 were all negative; the reaction was doubtful July 17, negative August 23, doubtful October 5, negative October 14, doubtful October 31, and negative November 14; cow aborted October 28; the calf lived a few hours only; the cow was sold for slaughter November 21. The reaction

[4] The writers are indebted to Dr. J. F. Shigley, associate professor of veterinary science, for veterinary service rendered; and to Dr. C. J. Marshall, State veterinarian, and to his associates in the Bureau of Animal Industry, for the performance of the agglutination tests on which this report is based.

of this cow was never positive. She aborted after having had iodine for three days, about four months after the first indication, by blood test, that she might have the disease. Three days after aborting the reaction was slightly suspicious, and two weeks later was negative. Because of the variable results of the blood tests prior to iodine feeding, the last change back to negative is of doubtful significance.

Cow No. 12 between October 30 and November 25 received 26.702 g of iodine; agglutination tests were negative throughout; the cow calved normally January 1; she tolerated 1 g of iodine a day and did not go off feed.

Cow No. 11 between October 30 and November 25 received 26.702 g of iodine; agglutination tests were negative throughout; the cow calved normally November 24; she tolerated 1 g of iodine a day for 26 days.

Cow No. 15 between October 30 and November 25 received 26.702 g of iodine; agglutination test were negative throughout; the cow calved normally January 1; she tolerated 1 g of iodine daily for 26 days.

Cow No. 16 between October 25 and December 12 received 49.662 g of iodine; agglutination tests were negative prior to October 14, and doubtful October 14 and 31. The reaction was highly suspicious November 14, positive November 19, and highly suspicious on December 13. The cow was sold for slaughter December 13, while carrying a calf, and was due to freshen February 10.

Cow No. 17 between October 19 and November 9 received 41.143 g of iodine; agglutination tests were negative prior to August 23, when the test was positive; the cow aborted August 27; the reaction was positive October 14 and November 14; the cow was sold for slaughter November 17. This cow tolerated about 2 g of iodine daily for 21 days with no apparent bad effects except that less than the usual amount of hay was consumed.

Cow No. 18 between October 19 and November 9 received 42.147 g of iodine; agglutination tests prior to August 23 were all negative; the cow aborted August 1; the reaction was positive August 23, October 14 and November 14; the cow was sold for slaughter November 17. Iodine feeding at the rate of 2 g a day for 21 days brought about no change in reaction to the agglutination test. The heavy dosage of iodine caused no apparent ill effects, except that the cow consumed less than the usual quantity of hay.

Cow No. 19 between October 10 and November 25 received 26.702 g of iodine; agglutination tests were all negative; the cow freshened normally April 19. One g of iodine daily for 26 days during the fifth month of pregnancy was tolerated with no ill effects.

Cow No. 21 between October 25 and November 12 received 17.88 g iodine; agglutination tests prior to October 14 were all negative; the reaction was slightly suspicious October 14, highly suspicious October 31, and positive November 14. The cow was due to calve November 24, but aborted October 31, when iodine feeding had been in progress 6 days, and was sold for slaughter November 17. The reaction changed from slightly suspicious to positive while the cow was receiving iodine at the rate of 1 g daily.

Cow No. 23 between October 30 and November 25 received 26.702 g of iodine; agglutination tests were all negative prior to November 19 when the reaction was highly suspicious. The cow aborted November 28, being due to calve February 10, and was sold for slaughter November 29. This animal was apparently infected and was incubating the disease before the iodine feeding was started. After being on the iodine (1 g daily) for 19 days she reacted in a manner designated highly suspicious, and aborted 9 days later, 3 days after the close of a 26-day iodine feeding period.

Cow No. 25 between October 30 and November 25 received 26.702 g of iodine; agglutination tests were all negative. The cow when examined on November 27 was found to be pregnant, and freshened normally on April 11. This cow (negative to abortion test) was fed 1 g of iodine daily for a 26-day period with no ill effects, in the fourth and fifth months of pregnancy.

Cow No. 26 between October 30 and November 25 received 26.702 g of iodine; agglutination tests were all negative; the cow aborted December 10; the calf was 6 weeks premature. This cow was sold for slaughter December 13. A blood sample taken on date of slaughter was found negative. The cow aborted 18 days after the close of a 26-day iodine feeding period. A positive blood reaction had not developed 3 days thereafter.

Cow No. 28 between October 19 and November 9 received 33.957 g of iodine; agglutination tests prior to July 17 were all negative, but were positive July 17 and August 23. The cow aborted September 12; the calf was born dead; the cow was due to calve in January. The reaction was positive October 14 and November 14; the cow was sold for slaughter November 17. She had aborted

about one month previous to a 21-day period of iodine feeding. Iodine feeding (1½ g daily) produced no change in reaction.

Cow No. 31 between October 19 and November 9 received 13.621 g of iodine; agglutination test August 23 was highly suspicious; and positive October 5, October 14 and November 14. The cow aborted October 4, the calf being dead, and was sold for slaughter November 15. This cow aborted two weeks previous to beginning of 21-day iodine feeding period and would tolerate only a small quantity of iodine.

Cow No. 32 between October 25 and January 1 received 48.662 g of iodine; agglutination tests were all negative until October 5 when the test was designated slightly suspicious; on October 14 it was highly suspicious, positive October 31, highly suspicious November 14, and positive November 19 and December 3. The cow aborted December 11, and had living calf weighing 45 pounds, one month premature, which lived until December 23. The reaction of the cow was positive December 17 and December 31. This cow was sold for slaughter January 7, 1930. The reaction changed from positive to highly suspicious about three weeks after iodine feeding was started, then changed to positive, and the cow aborted when the iodine feeding had been in progress 47 days. Iodine feeding was then continued 20 days longer with no change in the blood reaction.

Cow No. 34 between October 30 and November 25 received 26.702 g of iodine; agglutination tests were negative until November 19, when the cow reacted in a manner designated slightly suspicious; the reaction was positive December 3 and 13; cow aborted December 7, but the calf lived only 12 hours. The cow was due to calve December 27, and was sold for slaughter December 13. The blood test when iodine feeding was started was negative but changed to slightly suspicious when the cow had received iodine 20 days, and two weeks later the reaction was positive. Four days later the cow aborted, 12 days after the close of 26-day iodine feeding period.

Cow No. 35 between October 19 and November 25 received 40.303 g of iodine; agglutination tests were negative prior to May 1, on which date test was positive; they were also positive July 17, August 23, October 14, and November 14 and 19; the cow would not conceive and was sold for slaughter November 29. A 36-day iodine feeding period, in which a total of 40 g of iodine was fed, produced no change in the positive blood reaction.

Cow No. 37 between October 19 and November 9 received 42.639 g of iodine; agglutination tests were negative prior to July 17, on which date the reaction became positive. The cow was not pregnant. Tests were positive August 23, October 14, and November 14. The cow was sold for slaughter November 15. A daily dose of over 2 g of iodine for 20 days made no change in the blood reaction. The cow ate less than the normal quantity of hay.

Cow No. 38 between December 4 and December 12 received 18 g of iodine; agglutination tests were negative prior to April 15, on which date the test was positive; it was positive May 1, August 23, October 23, November 14 and 19, and December 13. The cow was due to calve January 8, but was sold for slaughter December 13. With eight days of iodine feeding, at the rate of 2¼ g a day, during the eighth month of pregnancy, there was no change in blood test.

Cow No. 39 between October 25 and November 25 received 31.662 g of iodine; agglutination tests were negative until October 5, on which date the test was slightly suspicious; it was negative October 14 and 31, and November 14, and slightly suspicious November 19. The cow was sold for slaughter November 29. This cow had an abscessed ovary and would not conceive. Her blood test was never more than slightly suspicious. Thirty-one days of iodine feeding (1 g daily) produced no change in reaction.

Cow No. 40 between October 29 and December 13 received 70.817 g of iodine; agglutination tests were negative until August 23, on which date the test was positive; it was also positive October 14 and November 14, highly suspicious November 19, and positive December 3 and 13. The cow was due to calve January 18, but was sold December 13. A 45-day iodine feeding period, in which a total of over 70 g of iodine were fed, produced no change of blood reaction.

Cow No. 41 between October 30 and November 25 received 26.702 g of iodine; agglutination tests were negative prior to November 14, on which date test was slightly suspicious, and again negative November 19. The cow was sold November 29 because of failure to conceive. Reaction was negative at the time iodine feeding was started, but slightly suspicious after the cow had received iodine for two weeks. Two weeks later the reaction changed back to negative.

Cow No. 44 between October 19 and January 1 received 82.755 g. of iodine; agglutination tests were negative until August 23, when the test was highly suspicious; tests were also highly suspicious October 5, 14, and 31, and November

14, positive November 19 and December 3, 17, and 31. The cow calved at full term December 19, and the calf was apparently healthy. The blood test on this cow was highly suspicious when iodine feeding was started, but changed to positive after the cow had been given iodine for one month, and continued positive until the cow was sold. Iodine was given, in doses of more than 1 g a day for 73 days including the last 60 days previous to a normal, full-time parturition.

DISCUSSION OF RESULTS

Iodine was first administered at the rate of 5 g of the element a day, in the form of iodized linseed meal. This quantity threw the cows off feed on the second day, but it was found that most individuals would tolerate from 2 to 3 g of iodine a day, at least for a limited period. Certain cows tolerated 2 g of iodine daily, as long as it was fed to them, while others would not tolerate more than 1 g a day. Some of the cows resented the presence of the iodized linseed meal in their ration from the beginning, while others paid little attention to it and appeared to eat their grain with the usual relish. After receiving a heavy dosage of iodine for several days, there was in all cases a decrease of appetite for hay. Possibly the iodine interfered with essential fermentations in the rumen.

After several days of heavy iodine feeding the cows became sluggish; the hair grew rough, and in certain individuals there was copious nasal discharge, and brownish discoloration of the skin about the eyes, nose, and tail.

The cream produced by the cows that received iodine was rejected by the inspector at the college creamery, because of its objectionable odor; and analysis of the cream, and also of the mixed milk of cows Nos. 5, 16, and 38, revealed the presence of iodine in very large quantities.

At the time this investigation was started it was the plan to continue the iodine feeding for several months, but conditions which developed thereafter rendered it desirable to dispose of all animals infected with abortion disease without delay. Two cows, Nos. 5 and 38, were fed iodine for only eight days. The average length of time during which the 23 others received iodine was 30½ days. Of these cows 6 received iodine for more than 30 days and the remaining 17 received iodine for an average of 23.6 days. The average total quantity of iodine fed these 17 cows was 31.7 g. Three cows, Nos. 16, 32, and 44, were fed for an average of 63 days, the average total of iodine given being 63.7 g.

THE EFFECTS OF IODINE ON THE GROWTH AND PHYSICAL CONDITION OF DAIRY CALVES

The object of this investigation was to learn whether iodine, especially in the form of iodized linseed oil meal, is of value in the rearing of dairy calves.

The calves which served as subjects were grade Holstein females which were purchased at ages of 1 to 5 days in groups of 4 to 12—as they were available—from dairy farmers in Bradford County. Great care was taken to select vigorous animals of uniform size, and they were reared in accordance with the usual practice on good dairy farms.

In the conduct of the experiment the identity of the groups of the calves as purchased was maintained, for convenience, each group being

divided into pairs, as evenly balanced as possible, one animal of each pair receiving iodine and the other serving as a control.

The calves received whole milk until they were 2 to 3 weeks of age. The ration was then gradually changed to skim milk, grain, and hay. The two calves of each pair were fed exactly the same quantities of feeds (with the exception of hay) throughout the experiment, the quantities allowed to each pair being determined by the calf with the smaller appetite.

The first four or five weeks' feeding constituted a preliminary period. During this time the calves were established on the experimental ration, and the feeding was carried out in the same systematic manner as during the later experimental feeding.

The grain mixture was made up of 75 pounds of oats, 75 pounds of wheat bran, 40 pounds of yellow hominy feed, 40 pounds of yellow corn meal, 50 pounds of linseed meal, and 1½ pounds of salt.

Four kinds of hay were fed, at different times during the experiment, viz, early cut timothy, high-grade New Jersey alfalfa, mixed hay of good quality, and high-grade red clover. The respective proportions of the total hay fed were 4, 15, 22, and 59 per cent. The timothy was fed during the preliminary period because of its usefulness in the prevention of digestive disturbances in young calves.

Each calf was given as much hay as it would eat. With each pair of calves, without exception, the one receiving iodine ate less hay than did the control.

The calves were weighed once each week, and a measurement of the height at the withers was taken once a month. The average daily intake of feeds and the total cod-liver oil and iodine fed are shown in Table 2.

TABLE 2.—*Average daily feeds and total cod-liver oil and iodine eaten by calves*

Group No., number of days on experiment, and subgroup	Average daily skim milk	Average daily grain	Average daily hay	Total cod-liver oil per calf	Total iodine per calf
	Pounds	*Pounds*	*Pounds*	*Cc*	*Grams*
Group 1, 139 days:					
3 calves receiving iodine	16.73	2.04	1.55	283.3	2.570
3 calves receiving no iodine	16.73	2.04	1.83	283.3	None.
Group 2, 131 days:					
6 calves receiving iodine	16.49	2.13	1.79	265.0	2.409
6 calves receiving no iodine	16.49	2.13	1.85	265.0	None.
Group 3, 117 days:					
4 calves receiving iodine	16.20	2.34	1.89	255.0	2.759
4 calves receiving no iodine	16.20	2.34	2.16	255.0	None.
Group 4, 110 days:					
3 calves receiving iodine	15.07	1.99	1.14	190.0	1.788
3 calves receiving no iodine	15.07	1.99	1.44	190.0	None.
Group 5, 103 days:					
2 calves receiving iodine	15.46	1.68	1.68	190.0	1.615
2 calves receiving no iodine	15.46	1.68	1.51	190.0	None.
Group 6, 75 days:					
3 calves receiving iodine	14.48	2.19	1.35	260.0	.727
3 calves receiving no iodine	14.48	2.19	1.31	260.0	None.

The analysis of the ration indicated a normal iodine intake considerably greater than the 1 mg weekly which Marine [5] reported as adequate for thyroid maintenance in dogs. This estimate of the iodine content of the ration disregards the analysis of one grain mixture, a sample of which showed one hundred times the amount of iodine present in other foods of similar type. Unfortunately the

[5] MARINE, D., and KIMBALL, O. P. Op. cit.

analysis was made after the feeding with the questioned material had been completed, and it was impossible to determine whether the extremely high iodine content was continuously present or whether the sample was unaccountably contaminated at the time of taking.

The quantity of iodine fed was adjusted once a week, the standard allowance being 30 mg per hundred pounds live weight. The iodine was fed once a day in the skim milk. After they had received iodine for about four weeks, the calves displayed marked evidence of having had too much of it. Their coats became very rough, and they did not eat with the usual appetite. The feces were thin and of an abnormal reddish-brown color. There was considerable variation in the tolerance of the individuals for iodine, and the appetites of some were so much affected that they ate only a small proportion of the normal quantity of hay. The administration of iodine, therefore, was discontinued for intervals of from 17 to 44 days, in the several groups, after which it was resumed at a lower level of intake, namely, 10 mg per hundred pounds live weight a day.

Table 3 gives the intervals of time during which iodine was administered or was withheld.

TABLE 3.—*Schedule of iodine feeding*

Group	Interval during which iodine was fed from beginning of experiment	Interval during which iodine was not fed	Final interval during which iodine was fed at diminished rate to end of experiment	Group	Interval during which iodine was fed from beginning of experiment	Interval during which iodine was not fed	Final interval during which iodine was fed at diminished rate to end of experiment
	Days	*Days*	*Days*		*Days*	*Days*	*Days*
No. 1	43	44	50	No. 4	34	34	22
No. 2	43	44	43	No. 5	28	33	30
No. 3	48	17	65	No. 6	6	43	27

The iodine was omitted from the feed of four calves before the end of the investigation, as indicated by the next to the last column in Table 4.

The very poor physical condition of some of the calves that had received iodine suggested the desirability of special measures for restoring them to health. Cod-liver oil was therefore used, both calves of each pair receiving the same quantity—usually 10 c c a day. The cod-liver oil was continued, with each pair of calves, as long as it seemed to be needed by the calf that had received iodine. The average length of time during which the calves were given cod-liver oil was 31 days, although 2 received it for 72 days, 4 received it for 58 days, and 6 received it for 41 days.

During the final period of administering iodine the drug was given to 11 of the calves in the form of potassium iodide, instead of iodized linseed meal, in order to reveal possible differences in the response of the animals to iodine in the two combinations.

On the lower plane of iodine intake there was no definite evidence of iodine excess, and during the brief period of comparison of iodized linseed meal and potassium iodide no differences could be detected of their effect on the calves.

TABLE 4.—*Gains of calves in weight, and in height at withers*

Group and number of days on experiment	Controls, which received no iodine				Calves which received iodine					
	Calf No.	Initial weight	Gain in weight	Gain in height at withers	Calf No.	Initial weight	Gain in weight	Gain in height at withers	Period fed iodine	Total iodine fed
		Lbs.	*Lbs.*	*Centimeters*		*Lbs.*	*Lbs.*	*Centimeters*	*Days*	*Grams*
Group 1, 139 days	77	94	197	21.5	76	92	205	24.0	93	2.458
	78	100	219	23.0	80	96	190	24.5	93	2.665
	79	105	218	22.5	81	108	199	24.0	93	2.587
Average		99.6	211.3	22.3		98.6	198.0	24.1		
Group 2, 131 days	83	115	216	24.5	89	120	208	23.5	86	2.774
	85	100	193	17.0	95	93	156	21.0	83	1.982
	87	115	191	25.0	84	113	211	28.0	86	2.676
	86	90	189	24.0	91	89	217	24.0	86	2.323
	94	106	215	25.0	93	110	216	23.0	86	2.665
	88	88	177	20.0	82	87	164	21.0	86	2.037
Average		102.3	196.8	22.5		102.0	195.3	23.4		
Group 3, 117 days	101	101	183	17.0	100	104	176	19.0	99	3.052
	77	100	193	18.0	99	93	191	18.0	99	2.416
	96	113	178	18.5	102	105	165	19.0	99	2.551
	98	116	203	20.5	103	118	219	22.0	99	3.016
Average		107.5	189.2	18.5		105.0	187.7	19.5		
Group 4, 110 days	112	93	152	20.0	106	90	140	18.0	53	1.090
	108	110	132	18.0	111	102	141	19.0	76	2.111
	110	128	147	18.0	107	110	151	17.5	76	2.164
Average		110.3	143.6	18.6		100.6	144.0	18.1		
Group 5, 103 days	120	100	128	11.0	118	98	131	14.0	68	1.531
	117	110	161	18.0	114	112	160	17.0	60	1.700
Average		105.0	144.5	14.5		105.0	145.5	15.5		
Group 6, 75 days	122	111	126	15.0	121	119	130	15.0	32	.758
	127	100	117	14.0	125	109	116	15.0	32	.671
	126	105	107	12.0	123	105	111	18.0	32	.752
Average		105.3	116.6	13.6		111.0	119	16.0		

The gains in weight of the calves, as recorded in Table 4, do not indicate a significant difference in growth between those that received iodine and those that did not. Among the 21 pairs of calves, in 10 cases the calf receiving iodine made the greatest gain. The total increase in weight of the 21 calves receiving iodine was 45 pounds less than the total gain of the 21 calves that received no iodine. The mathematical interpretation of the differences in gains in weight, by Student's method, revealed odds of 2.8 to 1 indicating that the calves which did not receive iodine had made the greater average gain in weight. These odds are not statistically significant.

A similar interpretation of the gains in height at withers gives results favorable to iodine in five of the six groups. The separate consideration of the 13 pairs of Groups 1, 2, and 3, which received larger total quantities of iodine than did the remaining groups, gave odds of 65 to 1 in favor of the indication that the calves receiving iodine made the greater gains in height; while the consideration of the entire 21 pairs of calves gave odds of 77 to 1 in favor of the same possibility. These odds are statistically significant.

The cause of the apparent stimulation of skeletal growth, as a result of the administration of iodine, was not revealed. This response of the calves receiving iodine is especially puzzling because they ate appreciably less hay than did those that received no iodine—the hay containing a very considerable part of the calcium of the ration.

While there is ground for question as to the iodine intake of these calves, on account of the excessively high apparent iodine content of the grain mixture fed, it appears that the tolerance of calves for iodine is less than that of sheep, swine, and chickens.

It also seems that the iodine of iodized linseed meal, which was fed during the greater part of the experiment, produced a more marked reaction on the calves than does the iodine of iodides.

At the time of this writing, the calves now being about 1 year of age, the difference in height of the calves which received iodine, as compared with those that received none, is no longer apparent, and that is no apparent difference in health of the calves as a result of iodine feeding.

THE VALUE OF SUPPLEMENTAL IODINE IN THE GROWTH OF SWINE

Forty purebred pigs of the Berkshire, Chester White, Duroc-Jersey, and Poland China breeds were divided into 10 lots of four each, as nearly as possible of the same size, sex, age, and breed representation.

Five lots were given iodine in addition to the basal ration (Table 5), while the other five were used as controls or checks.

TABLE 5.—*Live weights, gains in weight, and feed consumption of 10 lots of 4 pigs each, alternate lots receiving supplemental iodine with the ration for 126 days*

Lot and treatment	Average initial weight	Average final weight	Average daily gain	Feed per 100-pound gain
	Pounds	*Pounds*	*Pounds*	*Pounds*
No. 1, iodine	50.6	223.0	[a] 1.4	495.7
No. 6, check	48.7	221.0	1.37	426.7
No. 2, iodine	54.7	234.7	1.43	417.9
No. 7, check	55.2	221.7	1.32	447.7
No. 3, iodine	42.2	194.7	1.21	412.0
No. 8, check	40.5	165.7	.99	498.0
No. 4, iodine	41.2	188.0	1.16	335.5
No. 9, check	41.0	179.0	1.09	347.3
No. 5, iodine	51.5	188.7	1.08	345.5
No. 10, check	52.8	209.0	1.23	320.0

[a] Adjusted on account of the removal of 1 pig in the course of the experiment.

The composition of the rations is shown in Table 6. The rations were hand fed, the feeds being dry, to the limit of the pigs' appetites. The iodine was fed in the form of iodized linseed meal thoroughly mixed into the ration, once each day, at a rate of 50 mg of the element per hundred pounds live weight.

TABLE 6.—*Composition of basal ration of pigs*

Constituent	Proportions in which feed was mixed		Constituent	Proportions in which feed was mixed	
	First 112 days	Last 14 days		First 112 days	Last 14 days
Ground yellow corn	160	180	Tankage	20	20
Ground oats	40	40	Linseed meal	10	10
Wheat middlings	40	40	Salt	1	1

The age of the pigs at the beginning of the trial ranged from 10 to 12 weeks, and their average initial weight, in the several lots (Table 5), ranged from 40.5 to 55.2 pounds.

The test was continued until the average live weight per lot, in each group of five lots, reached approximately 200 pounds, which required 126 days with each group.

The initial and final weights, recorded in Table 5, are each the average of three weights taken on consecutive days. In addition, intermediate, individual weights were taken at 2-week intervals.

This experiment was conducted between December 3, 1929, and April 8, 1930. The pigs were confined in dirt lots in which there was no vegetation and were sheltered by small movable houses. It was possible, therefore, for the pigs to supplement the inorganic nutrients of the rations to a limited extent by eating earth.

No effects of the iodine feeding were observable in the performance of the pigs.

The essential data of the experiment are given in Table 5.

THE VALUE OF SUPPLEMENTAL IODINE IN THE FATTENING OF LAMBS

Two feeding experiments, one with 30 lambs fed individually by the paired feeding method, and a second with 171 lambs fed in six approximately equal groups, were conducted to learn whether iodine, in the form of iodized linseed meal, is of value in the fattening of lambs.

LAMBS FED BY THE PAIRED-FEEDING METHOD

The lambs were raised in northern Pennsylvania and were divided into 15 pairs, of the same sex, and as nearly as possible of the same weight and of the same condition as to fatness.

Each lamb was fed in the same individual crate throughout the entire experiment, which was started December 31, 1929, and ended March 18, 1930, a period of 77 days. Both members of each pair were given the same quantity of feed; and if one of a pair refused a part of its feed, the ration of its mate was diminished in like proportion.

The ration was one which is commonly used for fattening lambs, and consisted of alfalfa hay and a grain mixture, the latter composed of 9 parts of coarsely ground corn, 1 part of linseed meal, and 0.5 per cent of salt. The analysis of this basic ration showed iodine to be present in amounts usually considered adequate for the maintenance of thyroid function. In addition one member of each pair was given 33 mg of iodine per hundred pounds of live weight, in the form of iodized linseed meal, the iodine being fed once each day thoroughly

mixed with the grain ration. An earlier effort to feed the same total quantity of iodine in two doses a week was unsuccessful, since it sickened the lambs. The daily feeding, however, produced no unfavorable effects.

The initial and final weights are averages of three weighings each, taken on as many consecutive days, and in addition the lambs were weighed each week. The initial weights of the lambs ranged from 56 to 72 pounds, and the final weights from 72 to 105 pounds.

The lambs were fed in a closed shed on a ground floor bedded with straw, some of which was eaten. During winter days, when the ground was covered with snow, the lambs were permitted to run outside for a few hours each day. The lambs were not treated for parasites. Several were slaughtered, and all of these, especially lamb No. 2, were found to be infested with the nodule parasite.

The numerical data of the experiment are presented in Table 7.

TABLE 7.—*Live weights, gains in weight, and feed consumption of 15 pairs of lambs, one individual of each pair receiving supplemental iodine with the ration for 77 days*

Pair and treatment	Initial weight	Final weight	Average daily gain in weight	Average daily ration	Feed consumed per pound of gain in weight
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*
No. 1:					
Iodine	63	96	0.43	2.74	6.40
Check	65	99	.44	2.76	6.25
No. 2:					
Iodine	63	72	.12	2.16	18.51
Check	69	90	.27	2.16	7.92
No. 3:					
Iodine	72	104	.41	2.66	6.41
Check	72	105	.43	2.67	6.22
No. 4:					
Iodine	60	73	.16	1.75	10.37
Check	60	69	.12	1.78	15.23
No. 5:					
Iodine	60	89	.38	2.51	6.68
Check	56	85	.38	2.53	6.72
No. 6:					
Iodine	66	96	.39	2.54	6.53
Check	64	90	.34	2.54	7.55
No. 7:					
Iodine	61	83	.28	2.49	8.73
Check	66	90	.31	2.49	8.00
No. 8:					
Iodine	62	82	.26	2.46	9.49
Check	59	83	.31	2.48	7.97
No. 9:					
Iodine	69	89	.26	2.46	9.47
Check	68	90	.28	2.47	8.65
No. 10:					
Iodine	64	87	.30	2.10	7.06
Check	61	74	.16	2.10	12.42
No. 11:					
Iodine	68	90	.28	2.28	7.97
Check	61	81	.26	2.28	8.77
No. 12:					
Iodine	59	85	.34	2.33	6.91
Check	67	92	.32	2.33	7.10
No. 13:					
Iodine	57	80	.30	2.45	8.23
Check	56	78	.28	2.45	8.59
No. 14:					
Iodine	59	77	.23	2.23	9.55
Check	56	77	.27	2.25	8.28
No. 15:					
Iodine	69	99	.39	2.77	7.13
Check	63	96	.43	2.77	6.48

LAMBS FED IN GROUPS [6]

The group-feeding experiment was conducted between November 2, 1929, and February 14, 1930. The lambs used in this experiment included native fine wools grown in southwestern Pennsylvania, native muttons from northern Pennsylvania, and western lambs grown in the State of Washington. The lambs from each source were divided into 2 groups of 28 or 29 each, and 1 group from each of the 3 sources was fed, in addition to the regular ration, 33 mg of iodine per 100 pounds live weight daily for 104 days. The iodine was fed twice daily in the form of iodized linseed meal thoroughly mixed with the grain ration.

The average initial weights of the lambs in the lots ranged from 52.3 to 55.9 pounds. The average final weights ranged from 71.6 to 91.4. (Table 8.) The ration consisted of mixed hay, hand fed twice daily, and a grain mixture of 9 parts corn and 1 part linseed meal likewise fed twice each day.

TABLE 8.—*Live weights, gains in weight, and feed consumption of six groups of lambs, three of which received supplemental iodine with their ration for 104 days*

Type of lambs, group, and treatment	Lambs at start	Lambs at end [a]	Initial weight (average)	Final weight (average)	Daily gain (average)	Feed intake per 100 pounds of gain			
						Shelled corn	Oil cake	Hay	Total
Native fine wool:	*Number*	*Number*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
No. 1, iodine	29	27	55.9	73.0	0.16	530.4	59.7	738.3	1,328.4
No. 2, check	28	27	55.8	75.9	.19	461.9	50.7	631.3	1,143.9
Native mutton:									
No. 3, iodine	29	27	52.3	71.6	.18	500.8	52.3	648.7	1,201.8
No. 4, check	28	27	53.1	74.4	.21	450.2	49.9	565.3	1,065.4
Western:									
No. 5, iodine	29	27	54.1	86.6	[b].26	403.5	44.8	491.9	940.2
No. 6, check	28	27	55.6	91.4	[c].28	385.0	41.5	467.8	894.3

[a] 1 or 2 lambs in each group were slaughtered in the course of the experiment for carcass studies.
[b] Adjusted on account of the removal of 2 lambs in the course of the experiment.
[c] Adjusted on account of the removal of 1 lamb in the course of the experiment.

The lambs receiving iodine cleaned up their feed less promptly than did those receiving no iodine.

THE VALUE OF SUPPLEMENTAL IODINE FOR CHICKENS

In the experiment to determine the effects of the administration of supplemental iodine to chickens during growth and early egg production, four groups of single-comb white leghorn female chicks, containing 160, 170, 184, and 187 individuals, respectively, were fed the experimental rations from the time of hatching to the age of 32 weeks.

Groups 1 and 2 received the same varied mash ration, the animal-protein components of which were fish meal, meat scrap, and dried milk. Group 2 received in addition iodized linseed meal in a quantity sufficient to provide 50 mgs of iodine per 100 pounds of chicken a day.

The mash ration for Groups 1 and 2 consisted of 40 pounds of yellow corn meal, 15 pounds of wheat bran, 15 pounds of flour wheat middlings, 10 pounds of alfalfa-leaf meal, 10 pounds of dried milk, 5

[6] This report is based on an experiment conducted under the direction of Associate Prof. W. L. Henning, primarily as a comparison of types of lambs as feeders.

pounds of fish meal, 5 pounds of meat scrap, 2 pounds of steamed bone meal, 1 pound of salt, and 1 pound of cod-liver oil.

Groups 3 and 4 received a mash ration similar to that fed to Groups 1 and 2, except that the fish meal was replaced by meat scrap, Group 4 receiving this mash ration by itself, and Group 3 the same plus iodine, given at the same rate as to Group 2.

For the first 8-week period the iodized linseed meal was fed in accordance with predetermined standard live weights;[7] and thereafter in accordance with the experimental weighings which were taken individually every four weeks.

In addition to the mash rations all groups received a half-and-half cracked corn and wheat scratch mixture beginning with the fifth week and continuing to the end of the experiment.

The four groups of chicks used in the experiment were hatched at weekly intervals as follows: Group 1, March 20; Group 2, March 27; Group 3, April 3; Group 4, April 10, 1930. All males were removed at eight weeks of age, the data reported here being for females only. The chicks were placed in brooding quarters when 24 hours old and were fed the proper mash ration. The same mash mixture was fed, ad libitum, in hoppers, during the entire course of the experiment; and the scratch grain mixture, given after the fourth week, was also fed in hoppers.

During the first 16 weeks, the birds were housed in a long type of brooder house, each pen of which is 12 by 20 feet. Each group had access to a sun porch 8 by 10 feet in size. At the end of the 16-week period they were moved into laying quarters and remained there until the conclusion of the experiment. At no time in the course of the experiment were the birds allowed out on range.

Cannibalism was not manifest in an unusual degree in any of the groups during the experiment, the infrequent occurrence of this habit being approximately evenly distributed among the four groups.

No difference was noticeable in the behavior or the condition of the four groups of birds at any time during the experiment.

The average growth, the number of birds culled at the age of 16 weeks, the mortality, and the average number of eggs laid, for each group are given in Table 9.

TABLE 9.—*The effect of iodine on the growth, mortality, and early egg production of chickens*

Group and treatment	Age	Birds in groups	Average body weight	Mortality		Average number of eggs laid to age of 32 weeks
				Per period	Total	
	Weeks	*Number*	*Grams*	*Per cent*	*Per cent*	
No. 1, no iodine	0	160	38			
	4	160	232	0	0	
	8	160	508	0	0	
	12	159	817	0.6	0.6	
	16	155	1,039	2.5	3.1	31
	20	[a] 131	1,274	0	3.1	
	24	122	1,462	5.6	8.7	
	28	114	1,531	5.0	13.7	
	32	109	1,589	3.1	16.8	

[a] 24 culled at age of 16 weeks.

[7] CHARLES, T. B., and KNANDEL, H. C. REARING CHICKS IN CONFINEMENT. Penn. Agr. Expt. Sta. Bul. 218, p. 11, Table III. 1928.

TABLE 9.—*The effect of iodine on the growth, mortality, and early egg production of chickens*—Continued

Group and treatment	Age	Birds in group	Average body weight	Mortality		Average number of eggs laid to age of 32 weeks
				Per period	Total	
	Weeks	*Number*	*Grams*	*Per cent*	*Per cent*	
No. 2, iodine	0	170	40			
	4	170	235	0	0	
	8	169	523	0.6	0.6	
	12	167	796	1.2	1.8	
	16	[b] 142	1,124	1.2	3.0	35
	20	138	1,368	2.4	5.4	
	24	130	1,516	4.7	10.1	
	28	125	1,656	2.9	13.0	
	32	121	1,674	2.4	15.4	
No. 3, iodine	0	184	39			
	4	182	211	1.1	1.1	
	8	181	450	.5	1.6	
	12	174	711	3.8	5.4	
	16	[c] 146	983	1.6	7.0	24
	20	138	1,221	4.3	11.3	
	24	135	1,468	1.6	12.9	
	28	134	1,585	.5	13.4	
	32	130	1,764	2.2	15.6	
No. 4, no iodine	0	187	38			
	4	187	209	0	0	
	8	182	451	2.7	2.7	
	12	179	718	1.6	4.3	
	16	[d] 158	970	1.6	5.9	24
	20	148	1,220	5.3	11.2	
	24	133	1,451	8.0	19.2	
	28	132	1,531	.5	19.7	
	32	123	1,729	4.8	24.5	

[b] 23 culled at age of 16 weeks; also 2 died.
[c] 25 culled at age of 16 weeks; also 3 died.
[d] 18 culled at age of 16 weeks; also 3 died.

Attention is called to the fact that Group 2, which received iodine, excelled Group 1 in body weight, after the twelfth week; but doubt is thrown upon the significance of this observation by the fact that Group 3, which received iodine, was excelled by Group 4 in body weight.

SUMMARY

Twenty-five grade Holstein milking cows which were infected with, or had been exposed to, contagious abortion were subjected to liberal dosage with iodine in the form of iodized linseed meal. The length of time of feeding the different individuals varied from 8 to 73 days, the average being 28.7 days. The average total amount of elemental iodine fed was 34.32 g per cow. The average daily dose per cow was 1.2 g. Individual cows varied in their ability to tolerate iodine from 0.65 g to 2.13 g a day. The maximum total amount fed any individual was 82.8 g in a period of 73 days.

Agglutination blood tests for abortion disease were made at 2-week intervals during the iodine feeding, monthly tests having been conducted previous to that time.

The administration of iodine did not affect the condition of disease, as indicated by results of the agglutination blood test. The disease developed in the normal manner, in five cows, the tests progressing from suspicious to positive during the time iodine feeding was in progress. There were no clear-cut cases of change in reaction in the reverse direction. In the case of five cows of the six that aborted during the iodine treatment, the development of the disease was clearly indicated

by the blood test. Six animals furnished rather conclusive evidence that intensive iodine feeding during the last six months of pregnance was not harmful. No cows were fed iodine during the first three months of pregnancy.

The milk and cream produced during heavy iodine feeding had an objectionable odor and contained very large quantities of iodine.

Forty-two calves were fed, by the paired system of feeding, in an effort to learn whether iodine, especially in the form of iodized linseed meal, is of value in the rearing of dairy calves.

The duration of the experimental feeding of the different pairs of calves ranged from 75 to 139 days. Twenty-six of the calves were fed for 117 days or longer.

The ration was made up of skim milk, grain, and hay, as in good average practice. Cod-liver oil was included in the ration of all calves during a part of the time.

Iodine was fed to one calf in each pair in quantities ranging from 10 mg to 23.5 mg a day. Thirteen individuals received 18.5 mg or more a day, as an average for the experiment.

Thirty milligrams of iodine per hundred pounds of live weight, in the form of iodized linseed meal, proved to be a heavier dosage than the calves could tolerate, as revealed by loss of appetite (particularly for hay), roughness of the hair, digestive disturbances, and an emaciated condition, after receiving iodine at this rate for from three to five weeks.

With few exceptions, the calves could tolerate 10 mg of iodine, in the form of iodized linseed meal, per hundred pounds live weight, with no harmful effect.

The calves that received iodine ate less hay and made appreciably less gain in weight, but greater gain in height, on an average, than did those receiving no iodine.

The addition of cod-liver oil to the ration of the calves, when they were in an emaciated condition as a result of excessive iodine intake, proved very helpful in bringing the calves back to a normal condition.

Among 10 experimental lots of growing swine, fed as matched pairs of lots, three lots receiving iodine made greater gains in weight than did their check lots, while two check lots made greater gains than did the paired lots receiving iodine. The differences in three cases were too small to be of possible significance.

The five groups that received iodine required an average of 401.3 pounds of feed to produce 100 pounds of gain in weight, while the five groups used as checks required 407.9 pounds of feed to produce 100 pounds of gain.

It is therefore evident that no certain beneficial results, either as to amount or cost of gain in weight, were derived from feeding iodine, in the form of iodized linseed meal, to growing swine.

Fifteen carefully selected pairs of native Pennsylvania lambs were fed for 77 days in a study of the value of supplemental iodine in the fattening of lambs.

One lamb of each pair received in addition to its ration 33 mg of iodine a day in the form of iodized linseed meal, while the other lamb constituted a control or check.

Among the 15 pairs of lambs, in 7 pairs the lamb receiving iodine gained in weight the more rapidly, while in the remaining 8 pairs the check lamb gained the more rapidly.

Of 165 comparisons between weekly gains in live weight of the pairs, 63 favored the lamb which received iodine; 69 favored the check lamb, and 33 comparisons were of neutral significance.

The average difference between the average daily gains of pair mates was 0.01 pound in favor of the check lamb.

These results show clearly that iodine as fed in this experiment produced no appreciable effect in the fattening of lambs.

The average daily gains in live weight of the lambs in the groups which received iodine were slightly less than those in the check or control groups, the difference ranging from 0.02 to 0.03 pound.

The feed required for 100 pounds of gain in live weight was greater for the lots that received iodine. The iodine fed groups of the native fine-wool lambs, the native mutton lambs, and the western lambs required 184.5, 136.4, and 46.9 pounds more feed, respectively, for each 100 pounds of gain than was required by the check lots.

There was, therefore, a slight deterimental effect from the feeding of iodine to these lambs, and this effect was greater with lambs from the State of Pennsylvania than with those from the State of Washington.

Four groups of single comb white leghorn female chicks, containing from 160 to 187 individuals each, were fed from hatching time to the age of 32 weeks to determine the value of supplemental iodine in relation to growth and early egg production.

Two of the groups received a normal mixed ration containing fish meal, while the others received a similar ration but with meat scrap replacing the fish meal.

The iodine was administered in the form of iodized linseed meal at a rate supplying 0.05 of iodine per 100 pounds of chicken a day.

No certain effect of the iodine on the growth, mortality, or egg laying of the birds was observed.

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